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Case Report

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The Role of Immunological Reaction and Pro Inflammatory Mediators in Acne Vulgaris Etiopathogenesis, Applications in Dermatology Practice

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Abstract

Introduction: *Acne vulgaris was not infectious-very common-chronic inflammatory-self limitting disease of pilosebaceous units. Affected approximately 85% of adolescent as physiological acne and 10- 15% was manifest as clinical acne. Four major factors were involved in the etiopathogenesis: follicular hyperkeratinization, increased sebum production, abnormality of microbial flora and inflammation process.*

Acne vulgaris inflammation process was divided into early stages (adaptive Th1 cell & innate immune complement to non spesific antigen) and late stages (innate & adaptive immune response to spesific antigen). Acne vulgaris dermal inflammation was not directly caused by bacteria in the dermis, but it was resulted from biologically active mediators that diffused from follicle. Pro inflammatory mediators in acne had no protective role but it had harmfull effect caused persistent inflammation, soft tissue destruction and scar formation, cell cycles control disturbances-cell survival&apoptosis, termination of differentiation & proliferation, hyperkeratinization, sebaceous glands hypertrophy and lipogenesis, pruritus, pigmentation.

Biological active mediators were produced in innate immune response by the binding of the PAMPs bacterial's to innate surface PPRs (TLR2 & TLR4) of the keratinocytes, sebocytes & phagocytes, through innate cell cytoplasm PPRs-NLRP3 inflammasome activation, through PAR2 gene activation, through FGFR2 of keratinocytes activation. Biological active mediators were also produced by adaptive immune response by the binding bacteria that had been recognized by APC (through MHC class II) to the CD4+ of naive T cells that activated Th1, Th2, Th17 and Treg.

Case Report: *5 cases of acne vulgaris 3rd to 4th grade in female, 20-30 years old that had been treated by combination of topical tretinoin 0,05% and flucinolone acetonide 0,025% for 4 weeks were reported. The result were good (decreased acne to 1st grade to no acne), no side effects were reported. Tretinoin 0,025% was choosen as maintain treatment.*

Discussion: *Treatment of choices for chronic inflammation in acne were anti immunological reaction/pro inflammatory antagonists drugs. Steroid (acted in NFkB pathway) and tretinoin (antagonist TLR2 and FGFR2) topical combination had good result. Tretinoin decreased comedo, sebum production & hyperkeratinization, normalized keratinization and differentiation, replaced disorganized collagen fibers & formed thicker-elastic epidermis (skin rejuvenation), decreased PIH and scar/keloid formation. Steroid decreased chronic persistent inflammation & scar formation and decreased erythema & irritancy that were caused by tretinoin.*

Keywords: Acne vulgaris, Pro inflammatory mediators-application in dermatology practise

Summary

Acne vulgaris was not infectious-very common-chronic inflammatory-self limitting disease of pilosebaceous units. Affected approximately 85% of adolescent as physiological acne and 10- 15% was manifest as clinical acne. Four major factors were involved in the etiopathogenesis: follicular hyperkeratinization, increased sebum production, abnormality of microbial flora and inflammation process.

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Biological active mediators were produced in innate immune response by the binding of the PAMPs bacterial's to innate surface PPRs (TLR2 & TLR4) of the keratinocytes, sebocytes, innate cells-mo nocytes,macrophages,neutrophyls,mast cells, NK (produced IL1α,

TNFα, IL1, IL2, IL4, IL6, IL8, IL10, IL12, IL1β, IL18, IFNγ, IL17 and Leukotrien B4/LTB4), through bacterials activation to innate cell cytoplasm PPRs NLRP3 inflammasome of sebocytes (produces IL1β-IL17, IL18-IFNγ, IL33, IL1α) and through activation of PAR2 gene of the sebocytes & keratinocytes (modulates inflammatory response & innate immunity, caused deffect of differentiation, enhanced lipogenesis & SREBP1 expression, induced cytokine IL8, TNFα-HBD2-matrix metalloproteinase MMP1-1-2-3-9-13), FGFR2 of kertinocytes (activated by IL1alfa, efect: cell cycles control disturbance, termiantion and differentiation, hyperkeratinization, sebaceous glands hypertrophy and lipogenesis). Biological active mediators were also produced by adaptive immune response by the binding bacteria that had been recognized by APC (through MHC class II) to the CD4+ of naive T cells that activated Th1, Th2, Th17 and Treg.

Anti immunological reaction drugs (the effectivity of some drugs were still in research): Acted in NFkB central pathways (steroids), Antagonist TLR2 (Isotretinoin), Acted in NLRP3-↓IL1β (Nitric Oxide), Decreased of expression of PAR2 gene-↓IL8-↓TNFα-↓neutrophyl activation- destruction of enzym metalloproteinnase & as TLR2 antagonist (tetracyline, doxicycline, minocycline, erytromisin, roxytromycine, clarytromycin), down regulated TLR2 & TLR4 receptors-↓ IL1α, TNFα, IL8(ALA PDT), antagonist FGFR2 (anti androgen, isotretinoin, BPO, tetracycline, erytromycine), IL1 targetted agents (Anakinra, rilonacept, canakinumab), TNFα inhibitor (etanercept, infliximab, adalimumab), Leukotrien B4 antagonist (zileuron), Leukotrien antagonist (montelucast), Immunotherapy (immunization-CAMPS was neutralized & prevented inflammation).

Case Report. 5 cases of acne vulgaris 3rd to 4th grade in female, 20-30 years old that had been treated by combination of topical tretinoin 0,05% and flucinolone acetonide 0,025% for 4 weeks were reported. The result were good (decreased acne to 1st grade to no acne), no serious side effects were reported. Tretinoin 0,025% was choosen as maintain treatment.

Discussion & Conclussion: The combination of tretinoin and steroid as topical anti immunological reaction (anti pro inflammatory mediators) therapy for acne chronic inflammation processes were rational and had good result. They acted synergis, finally down regulated cytokine production, decreased inflammation, soft tissue destruction and scar formation. Single topical tretinoin as long term maintenance treatment was good.

The local side effect of tretinoin such as erythema and irritancy could be prevented by steroid, and steroid side effect such as acneiform eruption, acne steroid, epidermal thinning, reducing collagen synthesis could be prevented by tretinoin.

Steroid (acted in NFkB pathway) and tretinoin (antagonist TLR2 and FGFR2) were easy to get, cheap and safe in combination for short term periods (4 weeks). Tretinoin decreased comedo formation, sebum production & hyperkeratinization, normalized keratinization and differentiation, replaced disorganized collagen fibers & formed thicker-elastic epidermis (for skin rejuvenating), diminishing PIH-inhibiting melanin trasferred (for skin brightening), decreasing the expression of gen P53 (anti keloids). Steroid decreased chronic persistent inflammation, prevented from hypertrophic scar and keloid formation, decreased erythema & irritancy.

Chapter I
Introduction
Definition
Acne vulgaris was a very common self limitting disease, that was seen primarily in adolescent. It was defined as a chronic inflammation of the pilosebaceous units [1-4]. Acne was not infectious [3].

Incidence
Acne vulgaris affected approximately 85% of young people [1,3,4]. The age of onset was at puberty, typically 12-15 years but could firstly appeared at 25 years old [1,4]. The peak insidence of acne vulgaris was in 17-21 years (17-18 years in female and 19-21 years in males) [4]. Acne vulgaris was more severe in males than females [1]. The lower incidence of acne vulgaris was in Asians and Africans [1]. Acne in Black Americans were less evident than white Americans [3].

Diagnosis & Clinical Manifestation
Acne vulgaris was characterized (diagnosed) by the formation of comedones (open/black head and closed/white head), erythemathous papules and pustules, less frequently nodules and pseudocysts (could been ruptured, reencapsulated, inflammed and formed abscesses), draining sinus tracts (round isolated single nodules and cysts coalesce to linear mounds), that was accompanied by scarring in some cases [3]. Acne vulgaris lessions were polymorphic due to inflammation process of acne lessions [4]. Comedones were the primarily lessions of acne, but they were not unique and could been found in other skin disease like senilis comedo [1]. The predilection of acne vulgaris were on the face, trunk, upper arms and buttock [1]. Seborrhea of the face and scalp frequently presented and could been severe [1]. Duration of lession. The duration of lession was weeks to months [1]. Season. Acne vulgaris was worsen at winter and fall [1].

Symptom. It was Itchy and pain especially in nodulocystic type [1]. Itchy was rare. It could been found in early phase of acne or in succesfully treatment cases. Itchy was caused by releasing histamine like substances that were produced by P acnes that were killed by treatment [4]. Pain was also rare, could been found in patients with nodule and sinus espescially on the trunk [4].

Classification
Fitzpatrick classified acne vulgaris as non inflammatory lessions (white head and black head comedos) and inflammatory lession (papules, pustules, nodules, cysts) [1]. Plewig and Kligman in 1975 classified acne vulgaris in 3 types: comedonal, papulopustular and conglobata [5]. Cunliffe classified the severity of acne vulgaris according to the number and types/kinds of the lession into 4 types of acne vulgaris : mild, moderate, severe and very severe acne vulgaris [4]. The types of lession were: comedones, papules/pustules, nodules/cysts/sinus tracts, inflammation and scarring [4]. This case report classified and judged the severity and after treatment clinical improvement of acne vulgaris based on Cunliffe classification criteria.

Laboratory examination
No laboratory examination were required [1]. In the majority of acne patients had normal hormonal levels [1]. If endocrine disorders were suspected (especially in patients who had clinical manifestation of hyperandrogenism like irregular menses, hirsutism, hoarse voice, alopecia androgenism), determined free testosterone, follicle stimulating hormone, luteinizing hormone and DHEAS to

exclude hyperandrogenism and polycystic ovary syndrome [1,3,4]. Recalcitrant acne could also been related to congenital adrenal hyperplasia (11β atau 21β hydroxylase deficiency) [1].

Etiopathogenesis

Acne vulgaris was multifacto rial disease of pilosebaceous follicles. The important patophysiology of acne vulgaris were follicular hyperkeratinization, increased sebum production, P acne colonization, Inflammation [1].
Four major factors were involved in the etiopathogenesis [1-4]:

1. Follicular hyperkeratinization and cornification of the pilosebaceous duct.

It was not been known whether the initial trigger for acne was seborrhoea or ductal hyperkertanization or both [4].
Several factors that might been important as ductal hypercornification/ follicular hyperkeratinization mechanism were [1-4]:

a. Abnormal response to androgen
b. Abnormal lipid composition of the ductal corneocytes (local deficiency of linoleic acid)
c. Local cytokine activity (IL1α)
d. Microbial factors

Androgen that quantitatively and qualitatively normal in serum stimulated sebaceous glands to produce more sebum, there was high sebum secretion rate [1]. Essential fatty acid linoleic deficiency were characterized by inducing follicular hyperkeratosis, impacting of corneocyte and decreasing epithelial barrier function, low level linoleic acid led comedogenesis [1,3,4]. The changing in sebum secretion or composition (could flourished microorganism growth that activated immune system) could led to release of IL 1α by follicular keratinocytes, which in turn could stimulated comedogenesis [1]. Pro inflamatory cytokine stimulated abnormal keratinocyte proliferation and differentiation revealed obstruction [1,2]. Follicular plugging was formed and would prevented the drainage of sebum and androgens [2]. There was follicle impaction and distention, formed comedos that were disrupted and ruptured, there was leakaged of follicular materials that induced inflammed lessions [2,4].

The early hypercornification of acne was not been initiated by bacteria, but later there were microbacterials grew and bacterial lipases converted triglycerides to free fatty acids, there was changed of sebum composition and diluted linoleic acid concentration that led hypercornification (follicular hyperkeratosis) and comedogenesis [1,4].

Comedones represented as the retention and hyperproliferation of ductal corneocytes in the duct [3,4]. There were accumulation of multiple corneocytes in the duct could been caused by either an increased in production of basal keratinocytes or failured of the keratinocytes to be expelled from the duct [4].

Ductal hypercornification hystopathologically was presented as microcomedones and clinically as blackheads and whiteheads [3]. There was a signifficant correlation between the severity of acne and the number and size of follicular casts in comedogenesis [3]. There was an increasing in proliferation of ductal keratinocytes of non affected and affected follicles. Hystologically microcomedos were found in normal nearby sites of acne and area that was affected with acne [3]. The primary abnormality that led to hypercornification was not been related to change in keratin expression, hypercornification and comedogenesis might been related to failure the ductal corneocytes to separate [3].

The primary changed in the sebaceous follicle in acne was an alteration in the pattern of keratinization within the follicle [1]. Normally keratinous material in the follicle was loosely organized. In ultrastructural level, there were many lamellar granules and relatively few keratohyaline granules. Comedo formation was firstly formed in the lower portion of follicular infundibulum (infrainfundibulum) [1]. The keratinous materials were denser, the lamellar granules were less numerous, keratohyalin granules were increasing and some of cells were containing amorphous materials (which were probably lipid). were generated during the process of keratinization [1]. Kinetic studies demonstrated that there was an increasing in cellular turn over in comedones [1].

Corneocytes frequently contained about 20 % water but they were varies markedly with age [4]. The swelling of the epidermis was caused by hydration, that followed prolong soaking of the skin, particularly in warm water, was familiar in most people. Corniffied epithelium of the sebaceous follicle became hydrated, that might increased sebum outflow resistance by reducing the size of the pilosebaceous ostium [4]. This obstruction was associated with a decreased in outflow of sebum [3]. Acute obstruction of a particular pilosebaceous duct might then occured and thus precipitated acne [4]. It explained tropical acne and pre menstrual acne flared [3].

Comedogenesis was also related to the potential importance of what was called the sebolemmal sheath [3]. It had been suggested that the excretion of products from the sebaceous gland was occured through an organized acellular tubular conduit-the sebolemmal sheath was produced by sebaceous duct cells. The rupture of this sheath might contributed to comedogenesis [3].

a. Abnormal response to androgen influenced hypercornification
The evidence was accumulating to propose that androgens (a male steroid hormone such as testosterone) might play an important role in comedogenesis [3]. The cells of pilosebaceous duct had androgen receptors and 5α reductase type I (enzym that converted testosterone to DHT) was also present in this cells [3].

Androgen were known to regulate the development of sebaceous gland and sebum production. Androgen might played indirect at the follicular hyperkeratinization was supported by some obsevations [1]:

1. Androgen receptors had been localized to the outer root sheath of the infrainfundibular region in the follicles.
2. The formation of follicular cast reduced in patients that was treated with anti androgen.
3. Each of the key enzyme involved in androgen metabolism had been identified in the follicles.

b. Abnormal lipid composition in keratinocyter duct influenced hypercornification
Follicular hyperkeratinization might related to a local deficiency of linoleic acid, production of IL 1α within the follicle or possibilly the effect of androgens (high sebum secretion rate) [1]. Low essential fatty acid linoleic caused:

- ✓ Inducing follicular hyperkeratosis / hypercornification [1,3] (which might parallel with the increased scale that was found in comedo) [3].
- ✓ Impacting of corneocytes that formed comedones [4].
- ✓ Decreasing epithelial barier function [1,3]. (which might made the comedonal wall more permeable to inflammatory substances) [1,3,4].

Membrane coated granules were probably more related to barrier permeability than cell separation and that were decreased in commedones [3].

In examination of polar lipids recovered from comedone showed that the acyl ceramides were contain only 6% linoleic acid among esterified fatty acids, compared with 45% in normal human epidermis Linoleic acid concentration was decreased in acne patients sebum [3].

Other lipids had been incriminated; there were free fatty acids, squalene, squalene peroxides, oleic acid, isopropyl niristate, liquid parafin, wax ester, ceramid, and linoleic acid. Low ceramides and low level linoleic acid essential fatty acid in ceramide had been blamed for inducing comedones [3,4]. There were correlation between lipid peroxidase levels (an oxidative degradation of lipids resulting in cell damage) and the size of comedos. There were low level of linoleic acid and high sebum level in acne patients [4]. UVA radiation in lipid substance composition (squalene, oleic acid, isopropyl nyristate, liquid paraffin) induced comedogenesis [4]. There was high sebum flowed that produced a local deficiency of vitamin A in the duct that induced ductal cornification [4]. Than the changing in sebum secretion or composition could led to release of IL 1α by follicular keratinocytes, which in turn could stimulated comedogenesis [1].

The primary site of the developing comedone in the sebaceous follicle in acne vulgaris was at the level of the infrainfundibulum [4]. It was proposed that at the time of cell division, when the sebaceous cells still had an contact with the basement membrane, they still had and access to circulating lipid, including linoleat [4]. Once sebum synthesis began, no further lipids were accepted from circulation, so that more sebum was synthezised per cell, and linoleate content would been diluted [4]. This linoleate content would been released at the time of final cell ruptured and incorporated into various lipids in proportion to be relative rates (linoleic acid was diluted by sebum and the concentration would been low), at which these lipids were being synthesized at the time of cell ruptured [4]. Linoleic acid was essential fatty acid that could not been synthesized by human cell tissue [3,4].

The pilosebaceous unit comprised an matured epiuthelium and developed sebocytes through which the hair and sebum passed. Anatomically the pilosebaceous unit was divided into smaller units infundibulum (acroinfundibulum and infrainfundibulum) and sebaceous duct [4]. The sebocytes rest on the basement membrane that were contiguous with the dermis and extending from this basal layer into the central part of the gland. The sebaceous gland was a holocrine gland, the secretion was the result of self destruction of the sebocytes. The nucleus was moved to the periphery of the cell. The cell then entered the pilosebaceous duct. The sebum was secreted, than it was moved up with desquamated corneocytes and presented microbes to the surface [4].

c. Local cytokine influenced comedo formation

Inteleukine 1α was found in comedo, it was important in comedogenesis and it was produced by keratinocytes of the duct [3]. It was proved by in vitro study. This effect could been blocked by Interleukine 1 antagonists [4] and the formation was totally disrupted by EGF (Epidermal Growth Factor) [3,4].

d. Microbial factors influenced duct hypercornification

Two studies had failed to incriminate bacteria in the initiation of comedones, and it was proved by the fact that there were no bacterias that had been shown in some early comedones. Ultrastructurally and cultures of some early non inflamed biopsy material that were taken from lessions were sterile [3,4].

P. acnes was not involved in the initiation of comedones but might been involved in the later stages of comedogenesis [3,4]. The early hypercornification of acne was not been initiated by bacteria, but later there were bacterias colonization that produced lipase that converted triglyserida to free fatty acid, and increasing sebaceous free fatty acids would been changed sebum composition and diluted linoleic acid concentration that led hypercornification (follicular hyperkeratosis), corneocytes impaction that formed comedo and decreasing epithelial barier function that increased permeability of comedonal wall [1-4].

Biopsy and culture of early non inflammed lessions had shown that 30% of these were without bacteria suggesting that ductal bacteria were not needed for initiation of cornification [3]. Electron microscopy of early non inflammed lessions that were taken from prepubertal and early pubertal individual had demonstrated few or no bacteria [3]. Quantification of bacteria from comedones suggested that follicular colonization might been unrelated to comedogenesis [3].

2. Increased sebum production.

Normal or abnormal androgens stimulated sebaceous glands to produce more sebum or there was end organ androgen hypersensitivity response in normal hormonal level of acne vulgaris that made bacterial and fungal were flourished [1,3,4]. There was much debated concerning the prime trigger to acne, it was the increased of sebum production or formation of comedones or both abnormality developed paralel in the same acne prone pilosebaceous follicle [4]. Lipid composition influenced comedones formation [4].

Sebum excretion increased in acne patient than normal people and the increasing of sebum exctretion was equally with acne severity [3].

Increased production of sebum in acne patients was explained as 4 possibilities [4]:

1. An elevated level of circulating hormone that was caused by an abnormal pituitary drive.
2. An abnormal increase in the production of androgen in the adrenal and gonad.
3. An end organ hyper response of the sebaceous glands to normal circulating level of hormone.
4. Combination.

In most of acne patients had no hormonal misfit. Most patients in clinic did not require investigations of sex hormones simply because the patients seem otherwise normal, they responded well to an

appropriate treatment reasonablly and thus did not need detailed endocrinological examination [4]. There was rare cases that acne female patients had clinical sign of abnormal hormonal level like excessively hairy, hoarse voice, irregular menses and they got on well with the men and could been pregnant. In this patient could been found an elevation levels of circulating androgens or an abnormal pituitary drive [4].

There was an end organ hyper response of the pilosebaceous unit to normal levels of circulating androgens. And it was supported by the finding that the sebaceous glands in acne prone areas function differently to those in non prone areas, so acne could been found only on the trunk and none on the face, or acne just on the face and none on the back and chest [4].

A connection between acne and high rates of sebum secretion was supported by at least 3 types of evidence [1]:

1. Children did not get acne during the age range from approximately 2-6 years, when sebum secretion was extremely low.
2. Average rates of sebum secretion were higher in individuals with acne than those without acne.
3. Treatment that reduced sebum secretion (such as estrogen, 13 cis retinoic acid) improved acne.

Increased sebum production was presented as patient`s seborrhoea (greasy skin) [3]. Active sebaceous glands were a prerequisite for the development of acne. Acne patients male and female, excreted on average more sebum than normal subjects, and the level of sebum secretion correlated reasonably well with the severity of the acne [3]. Sebaceous activity was predominantly dependent on androgens sex hormones of gonadal or adrenal origin [3]. Abnormally high levels of sebum secretion could been thus resulted from high overall androgen production or increased availability of free androgen, because of a deficiency in sex hormone binding globulin (SHBG) [3]. Equally they could involved an amplified target response mediated either through 5α reductase of testosterone or an increased capacity of the intracelluler receptor to bind the hormone [3].

Lawrence et al found that only 41% of the acne patients had free testosterone level above normal. Lucky et al measured a number of androgens and their precursors as well as, and found that 52% of non hirsute women with acne had at least one abnormal hormone level. Darley et al found high sebum testosterone in 26%, low SHBG in 45% and high prolactin in 45% of 38 woman with acne However 24% of the total had no hormonal abnormallity [3]. Peripheral androgen metabolism might been important for example increased androsterone metabolism had also been reported in normo androgenic females [3].

In some published papers, it would seem that androgenic hormonal balance was disturbed in 50-75% of female acne patients [3]. However, this had not been established that it was the critical factor, and at least a quarter of all cases remain unexplained [3]. The development of acne were simply related to systemic hormone levels. But in general, acne patient had not frequently had endocrine misfit [3].

The acne did not occur simultaneously on all susceptible sites was consonant with the finding that sebum secretion were varies from follicle to follicle. In acne patients, there were marked heterogenicity in individual follicular sebum excretion [3]. This suggested that certain follicles might been proned to acne [3]. An enhanced peripheral response should been considered as a factor in many subjects [3].

The possible role of increased 5α reductase of testosterone to its more active metabolite was indicated, both by the deminstration that sebaceous glands in acne prone regions showed abnormallity high 5α reductase activity in vitro, and by the finding of abnormally high ammount of 5 alfa androstenediols in the urine of female acne patients [3]. There were 2 forms of 5α reductase, type I and type II, and the type I 5α reductase was more relevant. The activity of type I 5α reductase in isolated sebaceous glands also supported the end organ hyperresponsiveness theory for acne [3]. Androgen action on the sebaceous gland might been independent from serum hormone levels [3].

There was possibility that other hormones affected the sebaceous glands, either directly or by enhancing their response to androgens [3]. Low sebum excretion rate was low in individuals with isolated growth hormone deficiency, but this endocrinopathies was rare [3].

Sebum consisted of mixture of squalene, wax and sterol esters, cholesterol, polar lipid and triglyserides. As the sebum moved up the duct, bacteria especially *P acnes* hydrolized the triglycerides to free fatty acids, which eventually appeared at the skin surface. Free fatty acid fraction of the sebum was considered to be important in the causation of inflammation [1,3]. Triglycerides fraction in sebum was probably responsible for acne [1].

The role of individual lipid components in causing acne was uncertain. Lipid might been involved in ductal hypercornification or might been essential to the growth of bacteria [3]. Sampling of skin surface lipids had shown that patients with acne tended to have higher levels of squalene and wax esters, and lower levels of essential fatty acids linoleic acid, and a more frequent occurences of particular free fatty acids [3]. Linoleic acid was significantly reduced in ductal hypercornifications [3]. Linoleic acid levels were signifantly decreased in acne patients and there was inverse relationship between sebum secretion and linoleic acid esential fatty acid concentration of sebum [1]. Linoleic acid could not been synthesized in mammalian tissue and its concentration was diluted by subsequent endogenous lipid synthesis in sebaceous cell [1].

In was unclear, why elevated rates of sebum secretion led to acne. The triglyceride fraction of sebum, which was unique to humans, was probably responsible for acne. The bacterial population of the follicle hydrolized triglycerides to fatty acids, which eventually appeared on the skin surface. In the past, the free fatty acid fraction of sebum was considered to be important in the causation of inflammation, but in recent years it had become evident that there were probably other more important causes of inflammation [1].

The sebaceous glands produced a considerable amount of sebum in the first 3 months of life, which then gradually reduced to zero at 6 months of age. This neonatal stimulus was likely to be an effect of the fetal and neonatal adrenal androgens. After 6 months of age the sebaceous gkands remained quiscent until early adrenarche. At adrenarche, around 7-8 years, there was an increased in adrenal androgens, in particular dehydroepiandrostenedione, with the resultant increased in sebum excretion. In the early pubertal years

there was a further increased in adrenal androgens and gonadal androgen stimulus to the sebaceous gland. There was an obvious increased in greasiness of the skin (seborrhoea), even in subjects who did not have acne. The sebaceous gland was under endocrine control. The main stimulus to the sebaceous glands was androgens. The pituitary had an important role in controlling the androgen production via the adrenals and gonads. The adrenals in particular produced dihydroepiandrostenedione and the gonads in both sexes produced testosterone. The circulating androgens, in particular testosterone were bound to the sex hormone binding globulin and it was the 1-2% of free testosterone was dictated sebaceous gland activity [4].

In both sexes, independent of the presence or absence of acne, there was a gradual increased in sebum excretion from puberty and beyond reaching a peak at about the age of 16-20 years. Thereafter the level remained constant until there was a gradual decreased from about 40 years onwards in women and from about 50 years in males. In general, the sebum exretion rate (SER) in men was signifficantly higher than in women [4].

Patients with acne also had seborrhoea indeed many patients complained that as acne developed so there was an increased greasiness of the skin and on the scalp. There was a reasonable correlation between the amount of sebum production and the severity of acne. There was an evidence that those subjects with seborrhoea and acne had a higher number of sebaceous lobules per gland. Indeed one of the disappointing features of acne therapy with most therapies were the fact that despite an improvement in the acne the sebaceous was persisted. But in Dianette (cyproterone acetatate+ethinyl estradiols) and isotretinoin therapy, there was a significant reduction in sebum excretion and acne improvement [4].

Measurements of sebum excretion also showed that individuals with acne produced more sebum than individuals who had never had acne. There was a gradual decreased in sebum excretion beyond the age of about 40 years. Thus reduction in sebum alone was not accounted for resolution of acne [4].

There were differences of the lipid composition between the skin surfaces and in the sebaceous glands. Skin surface lipid composition had less triglycerides and more free fatty acids levels, and equally same levels of wax esther, squalene, cholesterol esters and cholesterol. It was caused by lipolityc enzymes that were produced predominantly by *P acnes* and *Staphylococcus epidermidis* that hydrolized triglycerides into free fatty acids when the sebum was moved up from the pilosebaceous duct [4].

3. The abnormality of the microbial flora.

Bacterial and *Pityrosporum ovale* `s lipase hydrolyzed triglycerides to free fatty acids that flourished bacterials and *Pityrosporum ovales* themselves [4]. There was lipid composition changing and free fatty acid would marked inflammation process [3,4]. Free fatty acid was comedogenic [4]. Bacterial and fungi were bound to the receptor of monocytes, keratinocytes, perifollicular and peribulbar macrophages, sebocytes, langerhans cells and other inflammatory cells (through TLR2 Receptor or others) and T lymphocytes (through CD4) than produced proinflammatory mediators (IL1α, TNFα, etc) that led to an inflammatory response [1,3,4,6]. P acnes induced TLR 2 Receptor expresion and play role in acne inflammation [6].

Adolescence and its attendance seborrhoea were associated with a significant increased in *P acnes* [3]. But there was a little or no relationship between the number of bacterias on the skin surface or in the duct with the severity of acne [3]. But in other books Cunliffe said that there was a correlation between the reduction in *P acnes* counts and the clinical manifestation of acne [4]. The development of resistance to *P acnes* might equated with clinical failure to treat the acne [4]. There was no P acnes colonization in non acne vulgaris patients [3]. *P acnes* colonization were at anterior nares [4]. And *P acnes* were important in acne pathogenesis [1].

Sebum excretion rate and ductal cornification correlated well with clinical severity [3].

Acne was not infectious [3]. The three major organisms were isolated from the surface of the skin and the duct of patients with acne were *Propinibacterium acnes*, *Staphylococcus epidermidis* and *Malassezia furfur*/*Pityrosporum ovale* [3]. There were three major subgroups of the propionibacterias: *P acnes, P granulosum* and *P avidum* [3]. Almost certainly *P acnes* and to lesser extended *P granulosum* were the most important [3] Nevertheless, as they lived in association with the *Staphylococcus epidermidis* and *Malassezia furfur*, three organisms had probably some control over the growth of P acnes [3]. And Staphylococci were the first organism that colonized the normal skin people [4].

Staphylococcus epidermidis were found as comensal (normal colony at nares, head and axilla) and patogen (as chronic nosocomial infection that infected through contaminated stuff in cardiac cathetherization or other procedures). It was difficult to be eradicated, it had high resistances, it was easy to be infected again after it was treated (by hands or contaminated stuff) and it was clinically found as chronic infection. But this colonization inhibited *Staphylococcus aureus* virulencies [7].

Pityrosporum ovale was lipophilic, saprophytic, budding, unipolar, dimorphic gram positive, double walled, oval to round yeast. They were normal part of the follicular skin flora, and alteration in flora caused uncontroled growth of yeasts and would been pathogenic [4]. They needed free fatty acid for survival (they had lipase that hydrolized triglyceride to free fatty acid). They were found in stratum corneum and in pilar follicles in areas with increasing sebaceous gland activity such as chest and back [8].

P acnes were gram positive, non motile, rods that tended to be irregular when the first isolated - some were short branching and required free fatty acid to colonize [3,4]. *P acnes* should been clumped, free fatty acid aided clumping, and so bacterial lipases might been necessary for clumping and for ductal colonization [3]. Isolates required 7 days of incubation under an aerob conditiom in 35-37°C (but this organism was not strickly an aerob) [4]. The physiological microenvironment of the follicle and the microenvironmental adaptation of *P acnes* might been important factor in the penetration of this non motile bacterium into the follicle duct [4]. They grew optimum at 30-37°C (temperature in the follicle) [4].

The environment of the bacteria was probably more important than their absolute number for development of acne lession [3]. In vitro, it had been shown that low oxygen tension, acidic pH (3,6-6,7) and nutrient supply [nitrogen, carbon, hydrogen, carbohydrate, amino acid, minerals, vitamin (biotin, nicotinic acid and thiamin) markedly

affected the growth of *P acnes* and the bacterial of active substances production such as lipase, proteases,hyaluronate lyase, phosphatase and smooth muscle contracting substance [3,4].

In the presence of light at high oxygen concentrations, *P acnes* grew well, but later the growth was inhibited because of photodamaging reactions involving excess oxygen and the endogenous microbial porphyrins [3].

The development of acne vulgaris was likely linked with the *P acnes* and very occasionally with the transient flora that were involved in acne (The transient flora was gram negative bacteria that was shed from anterior nares onto the adjacent skin after the resident flora was supressed by long term systemic or topical antibiotics) [3,4]. The limitted species of organism (resident organism) colonized the skin surface, such as propionibacteria, staphylococci, aerobic coryneform bacteria and the yeast *Malassezia furfur* [4]. Some microorganisms were appeared and dissapeared from the skin environment and constitued transient flora [4].

4. Inflamation processes.
Cunlife reported that hystologically CD4+ T lymphocytes were found in eatly 6 hours papular acne inflammation, CD4+ T lymphocytes and neutrophyl were found in 24-48 hours, CD4+ T lymphocytes, macrophage and giant cells were found in 72 hours [4].

Affected & Predisposed Factor

Several factors that affected, predisposed, triggered, influenced, exacerbated or aggravated acne vulgaris were: genetic, racial, atopic, seborhoeic, mestruation, hormonal misfit, diet, environment. ultraviolet light, hot and humidity, sweating, friction, occupation, stress, cosmetic, pomade genetic [4]. There were multifactorial genetical background and familial predisposition that had been proved in twin study [1,3]. Acne was polymorphous dermatosis with a polygenetic background, that did not follow Mendelian rules [4]. Most individual with cystic acne had parents with a hystory of severe acne [1]. Several study had shown that genetic factor influenced susceptibility to acne [3]. There were 45% acne parents`s in schoolboys acne patient in Germany and were also supported by genetic study in twins [3,4]. Besides genetic factor, the exogenous factor also influenced the severity of disease inflammation process, for example bacterial colonization [4]. Severe acne might been associated with XYY syndrome (rare) [1].

Racial. Acne in Black Americans were less evident than white Americans. Americans had more severe acne than Japanese [3]. Acne vulgaris was lower incidence in Asians and Africans [1].

Atopic.There was decreased incidence of acne vulgaris in atopic dermatitis patients that had low sebum production [3]. Seborrhoeic. Seborrhoeic dermatitis was concomitantly found with acne vulgaris in some cases, but the relationship had not been known [1].

Menstruation. About 70% patient reported 2-7 days premenstrual flared up of acne vulgaris related to sebaceous pores size changing that influenced the hydration of pilocebaceous epithelium [3,4]. There was an alteration of progesterone and estrogen levels [3,4]. Estrogen therapy increased SHBG and reduced free testosterone so there were decreasing of sebum production and libido.

The orifice of pilosebaceous duct was smallest between days 16-20 of the menstrual cycle. It reduced the flowed of sebum, produced relative obstruction and so increased the possibility of pro inflammatory cytokine mediators to concentrate in the lumen of sebaceous glands duct, thus stimulated the flare of acne premenstrually [4]. There was premenstrual changing in hydration of pilosebaceous epithelium and variation in sebum excretion during pre menstrual cycle that flared acne [3].

Testosterone was produced by ovarium and adrenal gonad, testosterone than converted to estrogen and progesterone. Testosterone levels peaked at the middle of menses phase (was around of ovulation) and there was increased libido. Testosterone was converted to DHT by 5α reductase enzyme. Testosterone and DHT were androgen that stimulated and were binded to androgen receptor in the sebaceous gland thus stimulated sebum production [1].

Most of acne female had normal menstrual cycle and normal hormonal level [4].
Hormonal misfit. In 24% acne vulgaris patient had no hormonal abnormality [3]. Most acne patients had normal hormone levels or levels at the upper end of normal range [3]. Most acne patients had no hormonal misfit, and had no need to investigate the hormonal problems in female patient [4].

Active sebaceous glands were required for the development of acne vulgaris [3]. Sebaceous activity was predominantly depended on androgen of gonadal and adrenal origin [3]. In the normal level of androgen production, there were increasing stimulation of sebum production in sebaceous gland of acne vulgaris patient [1]. There was also a possibility of an end organ hyper response of the pilosebaceous glands to normal circulating levels of androgen hormones [4]. And it was supported by the finding that the sebaceous glands in acne prone areas function differently to those in non prone areas, so acne could been found only on the trunk and none on the face, or acne just on the face and none on the back and chest [4].

Acne vulgaris patient extended on average more sebum than normal subject, the level of secretion was correlated with the severity of acne [3]. Androgen hormone had pro inflammatory effect, so androgen levels and antiandrogen therapy influenced acne inflammation severity [4].

There were rare cases with excessive androgen production of ovarium, adrenal and pituitary that were found in some exceptional case like acne in children (5-7 years), individual who poorly responded to 3 course oral isotretinoin acne therapy, patient with clinical skin androgenic manifestations like excessive hair, hoarse voice, irreguler menstruation, could not got on well with the men, could not been pregnant and female pattern alopecia (in polycystic ovarian syndrome and congenital adrenal hyperplasia) [4].

Diets. Cunliffe said that overall dietary factor did not cause acne [4]. In study proved no correlation between acne severity and whatever food ingestion [4]. In personal study there were no link between acne severity, calory intake, carbohydrate, lipids, protein, minerals, amino acid and vitamin [4].

But the possible effect of nutrition on the age of puberty might been relevant, as acne was more likely occured after the started of sexual development and this occured when the body weight attained about 48 kg [3].

The insidens of acne was low in people who had eaten rich fish diets and that was markly increased acne insidens in people who had eaten western diet with saturated fat [3]. It could been due to genetic factor [4]. Environmental factor also influenced the kind of people diet [3].

Chocholates, caramels and fatty acids were accused of aggravating acne [4]. High glycemic diets aggravated acne [2]. Chocholate had insuline like substance [2]. In high insulin levels, there were low SHBG levels and high free testosterone levels that increased sebum production. Insulin might affected SHBG, thereby influencing androgen clearance [3]. There was an inverse relationship between the serum levels of insulin and SHBG in woman. In obesity there was raised insulin levels, lower SHBG levels & total testosterone in both sexes [9]. Lower SHBG resulted in increasing of free testosterone, the effect of high free testosterone levels resulted masculinization and high sebum production. Estrogen therapy increased SHBG and reduced free testosterone so there were decreasing of sebum production and libido [9].

However post meal transient hyperinsulinemia did not play a role in hyperandrogenaemic acne patients [3]. And in study, high chocholate diet did not modulate the natural course of acne [4]. Chocholate appeared to have no significant influence in acne course study [3].

Reduced skimmed milk diet with calsium and vitamin D suplementation were benefit in acne patient and obesity [2]. Continuous low calori intake such as in anorexia nervosa patient and in patient with crash diet might improved the disease, and there were reducing of sebum excretion rate, changing sebum composition, decreasing sex hormones such as DHEA that explained clinical improvement of acne [4]. Dietary restriction resulted a mark weight loss and reduced seborhoea, but it could not been considered as routine treatment for acne [3]. Crash diets that were combined with strong physical stress could increased androgen release [4].

Environment. Acne insidence increased in people who migrated from east to western countries because of the dietary changing (rich fish diet to saturated fat diet), due to environment factor that influenced the people diet [4].

UV radiation. UV radiation was known to have wide ranging effect on cellular immunological system, but controled study on the therapetic effects were lacking [4]. UVA could converted squalene into squalene peroxidase which could enhanced comedogenesis [4]. But the other UV radiation like UVB, visible light (blue and red) and natural light 400-450 wavelength were beneficial to improve acne lession [4]. Artificial UV radiation appeared to be less satisfactory than natural radiation [3]. UVB produced tanning of the skin that produced camouflage that led to a subjective improvement of acne [4]. Erythemathous and suberythemathous dose of UVB could led to scalling of the interfollicular epidermis and might helped corneocyte desquamated from around of acroinfundibulum [4]. Narrow band UVB particularly helped in eczema and psoriasis [4]. The wavelength 400-450 nm could activate porphyrins (in the bacteria) that were produced by P acnes and could helped to destroy P acnes in the acne follicles themselves [4]. Visible light in both red and blue light range had been shown to improve acne as effective as benzoil peroxide. It was suggested that red light was antimicrobial [4]. Photodynamic therapy was under invenstigation [4].

Sweating. Up to 15% patient noticed that sweating caused a deterioration in their acne, especially if they lived or worked in a hot humid environment, for example as a cook. Ductal hydration might been a responsible factor [3].

Hot and Humidity. Acne could been worsen dramatically if patients were exposed to tropical and subtropical climates [4]. The holiday to humid environment frequently precipitated acne [4]. It might related to increase poral occlusive effect of skin hydration [4]. Excess humidity aggravated acne by an effect on sebum outflow [4].

Friction. Friction might contributed additional acne by irritating the upper parts of pilosebaceous duct [4].

Occupation. Hydration of the ductal stratum corneum induced acne in such occupation like catering [4]. Acneiform oil folliculitis and chloracne were the occupational acne [4].

Stress. It was unlikely that stress alone induced the formation of acne lession [4]. However acne itself induced stress and picking of the spot would aggravated the appearance [4]. That was particularly obvious in young females who presented acne excoriee [4]. Questionaire study had shown that many patients experience shame (70%), embarrassment and anxiety (63%), lack of confidence (67%), impaired social contact (57%) and significant problem with unemployment [4]. Severe acne might been related to increase anger and anxiety [4]. There were psychological and social effect of acne in inducing anxiety, depression and impaired the quality of life [4].

Cosmetic. It had shown that some cosmetic contained lanolin, petrolatum, certain vegetables oil, butylstearate, lauryl alkohol, oleic acid, isopropyl myristate, propylene glycol, D and C red dyes were comedogenic [3].

Pomade. Pomades were comedogenic greasy preparation [3].

Course & Prognosis

Acne vulgaris frequently cleared spontaneously by the early twentieth but could persisted to the fourth decade or older [1]. Treatment for acne might only required for 3-4 years, but the many patients with obvious clinical acne therapy would required for 8-12 years until the acne went into spontaneous remision [4]. Spontaneous remission frequently was around the age of 25 years, 93% of acne cases were resolved within 25 years and in 7% acne could persisted well into the mid forties or early fifties (up to the age of 45 years) and they were called as mature acne [4].

Inflammed lessions developed dynamically, with the majority exhibiting polymorphic clinical and hystological appearance before resolving [4]. Papule might become pustular before resolving, ussually through the macular phase [4]. Over 50% of superficial lessions were resolved within 7-10 days, whereas the deep seated nodules and pustules might persisted for 10-30 days or even longer [4]. The lessions would been healed and exacerbated by many factors and made acne vulgaris as one of the chronic pilosebacebaceous disease [1,3].

Flares occured in winter and with the onset of menses [1]. Several factors that affected, predisposed, triggered, influenced, exacerbated or aggravated acne vulgaris were genetic, racial, atopic, seborhoeic, mestruation, hormonal misfit, diet, environment. ultraviolet light, heat and humidity, sweating, friction, occupation, stress, cosmetic, pomade [4].

The squele of acne was scarring that might been avoided by early treatment, espescially with oral isotretinoin early in the course of the disease [1]. Early recognition and treatment of acne were important to prevent physical scarring espescially in inflammatory acne that could caused many psychological distress [4].

Limitted studies suggested that resolution did not relate to reduction of sebum production or surface bacteria [3]. Pierard had shown that there was an individual sebaceous glands function at different rates in acne patients [3]. The resolution associated with spesific changed in these acne prone hypersecreting glands [3]. The relationship between ductal hypercornification, inflammatory mediators, changed in the host response and resolution was obscured [3].

Complication

Acne scars were acne vulgaris complication event with the excellent treatment available were performed [2]. Intense inflammation led scar formation [1]. Scars were frequentlly occured in cystic acne, but less severe lessions also formed scar [2].
There were 2 sorts of scarring [3,4]:

1. There was loss of scar tissue (ice pick scar, depressed scar, macular atrophic scar, perifollicular elastolysis)
2. There was an increased of collagen tissue (hypertrophic scar, keloid scar)

Pitted scars/ice pick scars were typically occured on the cheeks. Perifollicular elastolysis was predominantly occured on the back, chest, neck. Keloid could been seen along the jawline and chest [2,4]. Scar might improved spontaneously over 1 year or longer.3 The rare scars complications were calsifications [3].

Other complications from acne were [3]:

- ✓ Prominent residual hyperpigmentation which was especially happened in darker skinned patients
- ✓ Pyogenic granuloma formation which was more common in acne fulminans and in patients treated with high doses isotretinoin
- ✓ Osteoma cutis which was consisted of small-firm-papules resulting from long standing acne vulgaris
- ✓ Solid facial oedeme, which was a persistent form facial swelling that was an uncommon but distressing result of acne vulgaris or acne rosasea.

Antibiotics resistances were the commonest complication. It was due to prolong treatment of acne vulgaris that was needed because of the disease chronicity [3,4]. Given treatment combination, changed the dose and duration of antibiotic therapy, given oral isotretinoin and the other non antibiotics regimen could solved the resistances to antibiotic problems [3]. The rare long term treatment of acne with antibiotics complication was gram negative folliculitis [3]. The lession appeared on anterior nares and extended to adjancent skin [1]. The physician should changed the diagnosed if there were suddenly appeared pustules and nodules [1].

There 2 kinds of gram negative folliculitis lessions were [2]:
Multipel pustules that were based on wide inflammation areas. It was more frequent. The etiology was *Enterobacter* and *Klebsilla*.
1. Deep indolent nodules. The etiology was *Proteus*.
Bacterial cultures and sensitivity tests should been performed to decide the prompt treatment. Ampicillin and trimetoprim were the treatment of choice for gram negative folliculitis. Oral isotretinoin was choosen for the resistent antibiotics cases [2,3].

Treatment

Goal of therapy. The goal of therapy were: removed plugging of the pilar drainage, reduced sebum production, treated bacterial colonization and prevented from scarring [1,4]. The treatment might been required for 3-4 years, patient with obvious clinical acne required 8-12 years therapy until the spontaneous remission was occured. Spontaneous remission wouldl been occured in 25 years old [3,4] and 7% could persisted until mid forties-early fifties and were called as mature acne [4]. Early recognition and treatment of acne was important to prevent acne scarring that caused physiological distress [4].

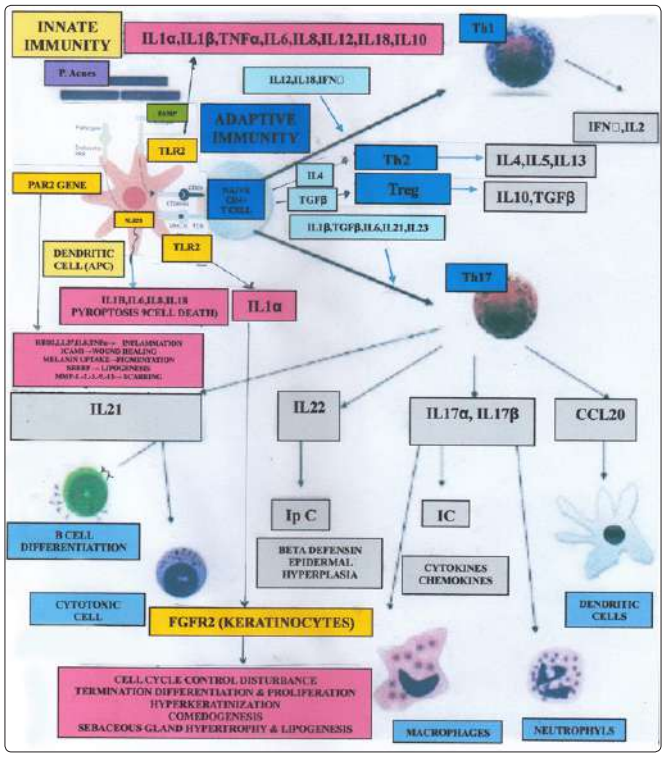
Treatment modality. The treatment modality were :

1. Topical therapy: topical antibiotics (Erytromycin, clindamycin), benzoil peroxide, sulphur, resorcinol, salisilic acid, retinoic,azeleic acid.
2. Oral therapy: oral antibiotics (doxycycline, tetracyclne, minocycline, amoxycillin, erytromycin, clindamycin, sulfa, dapsone) , oral isotretinoin [1-4].
3. Physical therapy: intralessional triamcinolone acetonide, cryotherapy, comedo extraction, cauterry, chemical peeling, photodynamic terapy, excision, surgical for severe and resistant cases [1-3].
4. Hormonal therapy had good result in normal or abnormal laboratory test patient. Spironolactone and cyproterone acetate treated acne vulgaris by reducing sebum production, reducing androgen excess and alleviating cystic acne [3,4]. Other hormonal therapy reduced sebum production by reducing testosteron level, but clinically had serious side effect (finasteride, flutamide, estrogen, gonadotropine releasing agonist and metformin).
5. Anti immunological reaction (anti inflammation): Blocked NFkB pathways (Corticosteroid), TLR2 Antagonist (Isotretinoin), Interleukine 1 antagonist/targeted agents (Anakinra, rilonacept, canakinumab), TNFα inhibitor (etanercept,infliximab,adalimumab), LTB4/ Leukotrien antagonist (zileuron, montelucast), Immunotherapy (immunization), Anti inflammation, Epidermal growth factor [1-3].

Treatment of complication. The complications were acne scar, persistent hyperpigmentation, pyogenic granuloma formation, persistent form facial swelling and could been treated by laser, chemical peeling, skin needling and rolling, dermabrasion, laser dermablation, cryopeeling, filler, punch graft, intralessional steroid and fluorouracyl [3].

The rare long term treatment of acne with antibiotics complication was gram negative folliculitis [3]. Ampicillin and trimetoprim were the treatment of choice. Oral isotretinoin was choosen for the antibiotic resistance cases [2,3].

The Role of Immunological Reaction and Pro Inflammatory Mediators In Acne Vulgaris Inflammation Process



Innate And Adaptive Immunity In Acne Inflammation Process

The immune system had to protect the skin against harmful microbial, chemical and physical insults. The activation of innate immunity provided the first rapid but non spesific response against those harmful attacts. The activation of adaptive immune system was more spesific due to immunologic memory. However, the activation of both innate and adaptive immunity were very early event in the formation of acne lessions [1,10-12].

But in acne lession the immune response was different. There was adaptive immune response to non spesific antigen that be involved in early stage of acne inflammation process, it could be found CD4+ T lymphocytes in early 6 hours comedos formation. Than developed innate and adaptive immune response to spesific antigen P acnes that were involved in later stages of acne inflammation [4].

Many immunological processes that contributed to the formation of the acne lessions took place at the vary state of the disease at the skin. Skin was an important component of the innate immune system, providing both physical barier and rapid cellular responses by keratinocytes, langerhans cells and other infiltrating inflammatory cells. There was an ability of innate and adaptive immune response to recognize microbial pattern and initiated immune response in cutaneous disorders [3,4,13,14].

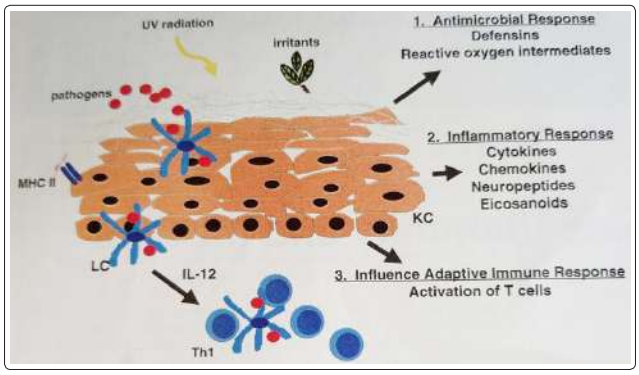
Recognition of microbial pathogen by the cells of the innate immunity (through the activation of TLRs2, NLRP3 by bacterias and PAR2 gene by proteases of bacteria) and adaptive immunity (that was activated by cytokines, spesific antigen P acnes and non spesific antigen gram positive bacterias), triggered host defence mechanisms to combated infection and prevented disease. However, activation of these pathways could also resulted in inflammation at the site of the disease and subsequent tissue injury. In acne, the host responses to P acnes resulted in the production of proinflammatory mediators that caused inflammation such as cytokines, chemokines, antimicrobial peptides, leukotriene B4, some enzyme like lysozime from leucocytes, metalloproteinnases, and proteases from P acnes, that caused severe inflammation, tissue destruction and scar formation [14,15].

Cytokine, especially IL1α than also activated FGFR2/Fibroblast Growth Factor Receptor2 pf the keratinocytes that caused cell cycles control disturbance and termination of differentiation, hyperkeratinization, sebaceous gland hypertrophy & increased lipogenesis that led to comedo formation and contributed in acne clinical manifestation [15].

Immune system was playing role in combating infection and in the pathogenesis of certain diseases state. In fact, various pathogens that the immune system attempting to fight, often played critical role in mediating the inflammatory response that led disease state. For examples group Aβ hemolytic Streptococcus in rheumatic fever, rheumatic heart disease and glomerulonephritis, Helicobacter pylory in gastritis and peptic ulcer disease, Chlamidia pneumonie in atherosclerosis, Pityrosporum ovale in seborheic dermatitis and P acnes in acne vulgaris. In all of that examples, infection by microorganisms itself was not the main caused of the disease, but rather the various inflammatory responses initiated by microbial agents that led to the distruction of the host tissue. Such responses included the formation of immune complexes, the recruitment and activation of of neutrophyls and monocytes, the released of cytokines and the released of degenerative enzymes [15].

In acne vulgaris, the released of pro inflammatory mediators/ biological active mediators like cytokines, chemokines, leukotriene, AMPs, enzymes (protease, lysozim) that triggered by P acnes unlikely to have protective role, but it had harmfull effect in acne by promoting inflammation and tissue destruction. TLR2, NLRP3, PAR2 gene, FGFR2 were a logical target for therapeutic intervention to block Pro inflammatory mediators like cytokines responses in acne and other inflammatory conditions in which tissue injury was detrimental to the host.

Innate Immunity In Acne Inflammation Process



Innate immunity was a defense mechanisms that were used by the host immediately after encountering a foreign ligand. These included the physical bariers such as the skin and mucosal epithelium, soluble factor such as complement, antimicrobial peptides, chemokines and cytokines and cells of the innate immune system including monocyte/macrophages, dendritic cells and polymorphonuclear leukocytes (PMNs) [1].

Innate immune responses in acne were mediated by a variety of different cell types including monocytes, macrophages, neutrophyls and dendritic cells as well as non immune cells such as keratinocytes and sebocytes. The possible contribution of P acnes in particular, in the activation of innate immune reaction had been demonstrated by several in vitro studies in keratinocytes, sebocytes and in peripheral blood monocytes [10,13,16].

The inflammation process in patients with acne was supported by polymorphonuclear neutrophyls, which produced a large number of free radicals by prostaglandins, leukotriene B4 and complement [17].

One of earliest signs of skin inflammation in acne was accumulation of CD68+ macrophages in the uninvolved skin of acne patients and in early stage (less than 6 hours old) lessions together with CD3+, CD4+ T cells resembling a type IV delayed hypersensitivity response. As the lession matures, the number of neutrophyls and CD8+ cells increased. After rupture of the follicular wall, the inflammatory response was mediated mainly by neutrophyls and later by macrophages and foreign body giant cells, It was possible but unussual for CD4+ T cells to accumulate in the inflammation site before neutrophyls [10,12,18,19].

Profesional antigen presenting cells (APCs) had not been extensively studied in acne. It had been noted that CD1a+ cells had reduced density in the epidermis and dermis around uninvolved follicles of acne patients. However in acne lessions in 6 hours and more, CD1a+ cells were found within the epidermis and follicle wall as well as in the periductal dermal infiltrates. CD1a was the major CD1 protein expressed especially on langerhans cells (LCs). FFAs, squalene and wax esters had recently been identified as autoantigens presented by CD1a and recognized by autoreactive T cells. Moreover HLA-DR expression was not increased within cellular infiltrates in uninvolved follicles of acne patients but in older lessions (6-72 hours). HLA-DR expression was increased in perivascular and periductal infiltrates including some basal keratinocytes. HLA-DR was one of the major histocompatibility complex (MHC) class II molecules, where antigen peptides fragments were bounds on on APCs and presented to CD4+ T cells. DCs, including LCs and dermal DCs, B cells and macrophages were the major MHC II expressing APCs [12,18,19]. HLA DR expression as Major histocompatibility complex class II molecules of APC was roled in adaptive immunity to spesific antigen in the later stages of acne lession (more than 6 hours).

Physical Barrier

Physical structures (skin and mucosal) prevented most pathogens and environment toxins from harming the host. Stratum corneum was the outer most layer of epidermis that resulted from the terminal differentiation of the keratinocytes, form the primary layer of protection from external environment. There was a layer of anucleated keratinocytes, that was composed by highly crossed linked proteinaceous cellular envelopes with extracellular lipid lamella consisting of ceramides, free fatty acids, and cholesterols. The free fatty acids create an acidic environment that inhibited collonization of some bacteria, but it flourished P Ovale and P acnes colonization in acne vulgaris patients [1].

Keratinocytes

Keratinocytes was the predominant cell in the epidermis, could mount an innate immune response through secretion of antimicrobial peptides (human β defensin 1,2,3 and cathelicidins) and through secretion of proinflammatory cytokines. Keratinocytes constituvely secreted very low level of cytokines. High level released of cytokines (IL1, IL6, IL8. IL10. TNFα) by keratinocytes were stimulated by injury or stimulation with exogenous factors such as lipopolysacharides (LPS), silica, poison ivy cathecols, Staphylococcus toxins and UV radiation. These cytokines induced differentiation and growth of the keratinocytes or other resident, induced migrating cell in the epidermis-dermis and vessels, acted as important mediators for local and systemic inflammatory and immune response [1].

Phagocytes

Phagocytes such as macrophages and PMNs were the major component of innate immune system and had capacity to detect the microbial pattern using complement receptor on their surface (PMNs phagocyted microbes coated with antibody and with complement active and alternative pathways. PMNs also had spesific receptor that could recognized pathogens directly. The mollecular pattern that should be recognized by phagocytes from the pathogen were lipopolisacharides from gram negative bacteria, lipothecoic acid of the gram positive bacteria, lipoproteins of the bacteria and parasites, glycolipids of the mycobacteria, mannans of the yeast, bacterial DNA sequences and double stranded RNA of the viruses [1].

Langerhans cell was the spesific innate immune cell in the epidermis, LC took up pathogen after encountering by endocytotic process and migrated to the daining lymphnodes where they developed to mature dendritic cell, and this process was stimulated by TNFα. Those migrating cells losed the ability to take up and process antigen but they upregulated MHC molecules and costimulatory mollecules to activate innate T cells. TNFα antagonists (drugs) could treat acne vulgaris by inhibited LC migration to the lymphnodes [1].

Neutrophyls were normally not present in the skin, however during inflammatory process, after stimulated by IL8 (a chemokines that had low mollecular weight that had chemotaxis effect), these cells migrated to the sites of infection and inflammation where they were the earliest phagocytic cells to be recruited, than neutrophyls degraded after pathogen (P acnes) phacytosis that was caused by lysosym enzymes that were produced [1].

Phagocytes expressed pattern recognition receptors (PPRs) that fucntion to be reconized pathogen asscociated mollecular patterns (PAMPs). Examples of the PPRs included mannose receptor that recognized mannan (yeast), CD14 that recognized Lipid A portion of LPS, surface Toll Like Receptors (TLRs) that recognized lipopolysacharides or peptidoglycans of the bacteria. PPRs were nonclonal receptor that were present on all phagocytic cells e.g. Macrophages, keratinocytes, sebocytes and did not dependent on immunologic memory because they were a germ line encoded (component of innate imunity) [1].

PAMPs were spesific to microbes and were not expressed in the host system. Innate immune systems could distinguish between self and non self PAMPs and relaying this messages to the adaptive immune response (in acne IL12 secreted by phagocytes in innate immunity stimulated Th1 responses in adaptive immunity) [1].

PPRs divided into several family of proteins by their structures, e.g. TLR and CD14 were leucine rich repeated. PPRs also divided into 3 classes by their function: secreted (that function as opsonin that binding microbial walls that than recognized by the classical

complement system and phagocytosis), endocytosis (exp. Mannose receptor that recognized mannan than mediating phagocytosis by macrophages) and signaling (signaling receptor activated signal transduction pathways that led to expression immune response genes like cytokines) [1].

Cytokines were secreted by macrophages in response to microbes were IL1, IL6, TNFα, IL8, IL12, IL18, IL4, IL10. IL1, IL6, IL10, TNFα were important in acute phase response response in the liver that inducing fever for the effective host defense. TNFα was the potent inflammatory response to infection, IL8 had chemotaxis effect in the site of infection, Monocytes produced IL12 (in innate immunity) that acted as important regulator of Th1 responses (in adaptive immunity) that essential for promoting CMI against intracellular microbial pathogens.

Most resistance problems were determined by Th1 and Th2 cell cytokines patterns [1].
Phagocytes secreted an inhibitory cytokine IL10, that inhibited CMI that was required for effective elimination of intracellular pathogens. In leprosy IL10 expression in lession corelated with susceptibility of infection. There was dysregulation of IL10 in human allergic diseases. IL10 was produced by malignant cells, where il10 was produced to evade the local immune response [1].

Langerhans cells, monocytes and macrophages presented antigen to T and B lymphocytes for initiation an adaptive immune responses (acted as APC-antigen presenting cells) [1].

Phagocytic cells of the innate immune system could also be activated by the cells of the adaptive immune system. CD40 was a 50kDa glycoprotein presented on the surface of B cells, monocytes, dendritic cells and endothelial cells, and its ligand was CD40L. CD40-CD40L interaction between T cells and macrophages played a role in maintenance Th1 types cellular response and mediation of inflammatory response. CD40-CD40L interaction in B cells activation, differentiation, up regulation and Ig class switching. Costimulatory activity induced on B cells then acted to amplify the response of T cells. These mechanisms were interplay between innate and adaptive immunity in generating effective host response [1].

Antimicrobial Peptides

Human β defensin located at the outer layer of the skin, produced by keratinocytes. Antimicrobial peptides had antimicrobial activity against a variety of microbes suggested that human β defensins were the essential parts of cutaneous innate immunity [1].

Antimicrobial peptides (AMPs) were evolutionarily conserved molecules, which protected the skin and other epithelial against pathogens and form a fast acting chemical barrier that also regulated the normal flora of the skin and mucosa. Several AMPs were induced in acne, which suggested that they influenced acne pathogenesis. However, it was not clear whether AMPs had a beneficial or detrimental antimicrobial effect by promoting inflammation [1,13].

In acne vulgaris, through the activatio of PAR 2 gene by proteases of P acnes, HBD1,2,3 were secreted and roled in inflammation. HBD1 roled in keratinocytes differentiation.

Keratinocytes, the predominant cells in the epidermis, could mount an immune response through secretion of antimicrobial peptides. Human epithelial cells produced β defensin, cystein rich cationic low mollecular weight antimicrobial peptides. Human β defensin 1 (HBD1) was constitutively expressed in the epidermis, was not transcriptionally regulated by inflammatory agents and had microbial activity against gram negative bacteria. HBD1 played a role in keratinocyte differentiation. Unlike HBD1, HBD2 transcription in keratinocytes was inducible by microbes including Pseudomonas aeruginosa, Staph. Aureus and Candida albicans, also stimulated by pro inflammatory cytokines TNFα and IL1. HBD2 also showed effective against gram negative bacteria such as Escherisia coli and P. Aeruginosa. HBD3 induced by TNFα and bacteria. HBD3 demonstrated potent antimicrobial activity against gram positive bacteria Staphylococcus aureus and vancomycin resistant Enterococcus faecium [1].

HBD attracted dendritic cells and memory T cells via chemokine receptors CCR6, provided a link between the innate and the adaptive immunity at the skin [1].

Other AMP called cathelicidins had also demonstated at the skin. Cathelicidins was an important component of innate imunity agains necrotic skin infections produced by group A Streptococci. These peptides were produced in increasing amounts following skin wounding due to their release by neutrophyls and increased synthesis by keratinocytes [1].

The AMPs human beta defensin 1 and 2 (HBD1 and 2), cathelicidine (hCAP18), psoriasin (S100A7), koebnerisin (S100A15), lactoferin, lysozyme, RNase7 granulysin, human neutrophyl protein 1-3 (HNP1-3) and neutrophile gelatinase-associated lipocalin (NGAL; the product of lipocalin 2 [LCN2] gene), were upregulated in acne lessions. Gene expression analysis of noninflammed skin of acne patients had shown that S100A7 and S100A9 were up regulated during isotretinoin treatment. In addition, P Acnes was shown to induce HBD2 and hCAP18 in sebocytes and keratinocytes in vitro [10,20].

Use of Janus Kinases and STAT Proteins in Signal Transduction by Selected Cytokine Receptors

Cytokine	Receptor Chains and CD Designations*	Jak Kinases	STAT Protein(s)
IFN-α, β	α (CD118), β	Jak1, Tyk2	STAT1, STAT2
IFN-γ	α (CD119), β	Jak1, Jak2	STAT1
IL-2	α (CD25), β_c (CD122), γ_c (CD132)	Jak1, Jak3	STAT3, STAT5
IL-3	α (CD123), β_c (CDw131)	Jak1, Jak2	STAT5
IL-4	α (CD124), γ_c (CD132)	Jak1, Jak3	STAT6
IL-4 and IL-13	α (CD124), α1 (CD213a1)	Jak1, Jak2	STAT6
IL-5	α (CDw125), β_c (CDw131)	Jak1, Jak2	STAT1, STAT5
IL-6	α (CD126), gp130 (CD130)	Jak1, Jak2, Tyk2	STAT1, STAT3
IL-7	α (CD127), γ_c (CD132)	Jak1, Jak3	STAT1, STAT3, STAT5
IL-9	α (IL-9Rα), γ_c (CD132)	Jak1	STAT1, STAT3, STAT5
IL-10	α (CDw210), β (IL-10Rβ)	Jak1, Tyk2	STAT1, STAT3
IL-12	β_1 (CD212), β_2	Jak2, Tyk2	STAT1, STAT3, STAT4
IL-15	β_c (CD122), γ_c (CD132)	Jak1, Jak3	STAT5
IL-17	α (CDw217)		STAT2, STAT3
IL-19	α (IL-20Rα), β (IL-20Rβ)		
IL-20	α (IL-20Rα), β (IL-20Rβ)		STAT3
	α (IL-22Rα), β (IL-20Rβ)		
IL-21	α (IL-21R), γ_c (CD132)	Jak1, Jak3	STAT1, STAT3
IL-22	α (IL-22Rα), β (IL-10Rβ)		STAT1, STAT3, STAT5
IL-24	α (IL-22Rα), β (IL-20Rβ)		STAT1, STAT3
	α (IL-20Rα), β (IL-20Rβ)		
GM-CSF	α (CD116), β_c (CDw131)	Jak1, Jak2	STAT5

Cytokine was a soluble mediators of immunity and inflammation were actively secreted by immune and non immune cells and they initiated, mediated and perpetuated inflammation and tissue damage [1,11].

Cytokine were a broad and loose category of small protein (5-

20kDa) that were important in cells signalling. Their released had an effect on the behaviour of cells around them. It could be said that cytokines were involved in autocrine signaling, paracrine signaling and endocrine signalling as immunomodulating agents. Their definite distinction from hormone was still part of ongoing research. Differentiate from hormones, that hormones were also an important signalling mollecules, in that hormone sirculated in less variable concentrations and hormone could be made by spesific kind of cell glands [11].

Cytokines was a category of immunoreactive protein, acted as humoral regulation which modulated the functional activities of individual cells and tissue at nano to picomolar concentration under normal or pathological conditions. The cytokines family consisted mainly of small water soluble proteins and glycoproteins. These proteins were secreted by immune cells and some other cells.

Cytokines included chemokines, interferon, interleukines, lymphokines and Tumor necrosis factor but generally not hormones or growth factors. Cytokines were produced by a broad range cells, including immune cells like macrophage, B lymphocytes and mast cells, as well as endothelial cells, fibroblasts and various stromal cells [1].

Cytokines (which included the large family of chemokines) were solluble polypeptide mediators that played pivotal roles in communication between cells of the hemopoitic system and other cells in body. Cytokines influenced many aspects of leukocyte function including differentiation, growth, activation and migration. Many cytokines were substantially upregulated in response to injury to allow a rapid and potent host response, cytokines also played important role in the development of immune system and homeostatic control of the immune system under basal condition. The growth and differentiation effects of the cytokines were not limited to leukocytes, but also mediated the growth and differentiation of other cells [1].

When cells and tissues in complex organisms communicated over distance greater than one cell diameter, soluble factor such as cytokines were required. A subset of this factors was produced or released transiently under infectious or injurious challenge to complex responses in the microenvironment of the tissue. Such response mobilized certain circulating white blood cells to the relevant injured area and guided other leukocytes particularly T cells and B cells, involved in host defense to specialized lymphatic tissue, remoted from the infectious lession but sufficiently close to contain antigens from the relevant antigen. After limited period of time, antibodies was produced by B cells and efector memory T cells could be released into the circulation and localized at the site of infection. Soluble factors produced by resident tissue cells at the site of injury, by leukocytes and platelets that were recruited to the site of injury, and by the memory T cells ultimately recruited to the area, all conspired to generate a evolving and effective response to a chalenge to host defense. The degree of this response should be transient, it was long enough to decisively eliminate the pathogen but short enough to minimize damage to healthy host tissues. The cell to cell communication involved in the coordination of this response was accomplished by cytokines [1].

The term cytokine was first decribed in 1975, it had distinct and easily recognizable biologic activities- IL1, IL2 and interferons (IFNs). Keratinocytes cytokines were first described in 1981 and the list of cytokines produced by this epithelial cells rivaled that of nearly any other cell type in the body.

There was primary and secondary cytokines according to cytokines pathways for its functions. Primary cytokines were those cytokines that could themselves initiated all the events required to bring about leukocytes infiltration in tissues. IL1 (α and β) and TNF (α and β) function were as primary cytokine. And the primary cytokines and other secondary cytokines (e.g. IL18) gave the signal through receptors that triggered the nuclear factor kappa B (NFκB) pathway [1].

IL1 and TNF induced cell adhesion molecule expression on the endothelial cells (both selectine and imunoglobulin super family members such as intercelluler adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). They induced a variety of cells to produce a host of additional cytokines and induced expression of chemokines that provided chemotactic gradient, allowing directed migration of spesific leukocytes subsets into the site of inflammation. Primary cytokines could be viewed as the part of innate immune system, and in fact, shared signalling pathways with the Toll Like Receptors (TLR)- a recently described family of receptors that recognized mollecular patterns characteristically associated with microbial products.

Many qualify as secondary cytokines, whose production was induced after cell stimulation by IL1 and/or TNF family mollecules. The term of secondary cytokines did not imply that they were less important or less active that primary cytokines, rather than their spectrum of activity was more restricted [1].

Cytokine acted through receptors, and especially important in the immune system. To mediate their effects, cytokines should first bind with the spesificity and high affinity to the receptors on the cell surface of responding cells. Many aspects of pleiotropism and redundancy manifested by cytokines could be understood through an appreciation of the shared mechanisms of signal tranduction mediated by cell surface receptors for cytokines. Most cytokines shared signals to cells through pathways (the central importance pathways were NFκB and Jak STAT pathway) that were very similar to those of other cytokines using the same class of the receptor. The fact that individual cytokines often use several downstream pathways of signal transduction account in part for the pleiotropic effect of these mollecules [1].

NFκB signal transduction pathway

There were extensive overlap between the biologic activities of the primary cytokines IL1 and TNF was the shared use of the NFκB signal transduction pathways. IL1 and TNF used completely disticnt cell surface receptor and proximal signalling pathways, but these pathways converged at the activation of NFκB transcription factor. NFκB was the cental importance in immune and inflammation processes because a large number of genes that elicited or propagated inflammation had NFκB recognition sites in their promoters. NFκB regulated genes include cytokines, chemokines, adhesion mollecules, nitric oxide synthase, cyclooxygenase and phospholipase A2 [1].

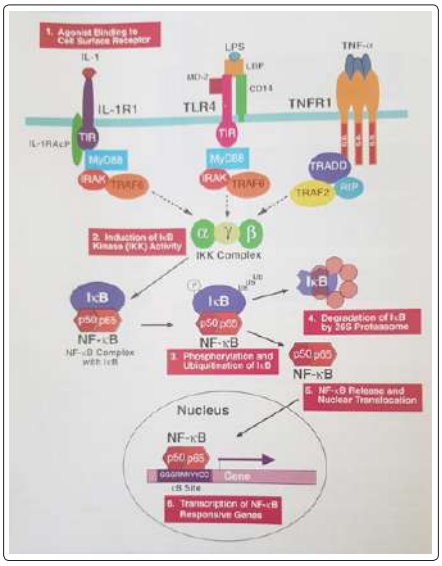
In non stimulated cells, NFκB heterodimers formed form P65 and P50 subunits were active because they were sequestered in the cytoplasm as a result of tight binding to inhibitor proteins in the 1κB family. 1κB than degraded by 26S proteasome complex, so there

were free NFkB (which contained a nuclear localization signal) was able to pass into the nucleus and induce expression of NFkB sensitive genes. Primary cytokines promoted inflammation by liberating NFkB from its association with 1kB.

Among the genes regulated by NFkB were IL1β and TNFα, which these cytokines had capacity to establish a positive regulatory loop that caused persistent infflammation [1].

NFkB pathway could activate by Proinflammatory cytokines (IL1 and TNF), bacterial products (e.g. Lipopolyscharides), oxidants, activator protein kinase C (e.g. Phorbol esters), viruses and ultraviolet radiation. Recently a cell surface receptor for the complex polysacharide (LPS), LPS binding protein and CD14 were identified as TLR4. The cytoplasmic domain of TLR4 was similar to that of the IL-IR1 (for IL1) and other ILIR family number and was known as TIR (for Toll/IL1 receptor). Ligand was bound to TIR domain, an adaptor protein called MyD88 was recruited, in turn than recruited one or more ILIR associated kinases (IRAK-1, -2 and -M), which then signal through TRAF6 (a member of TNF receptor associated factor family) to activated IKK complex, that than released NFkB and nuclear translocation in nucleus.

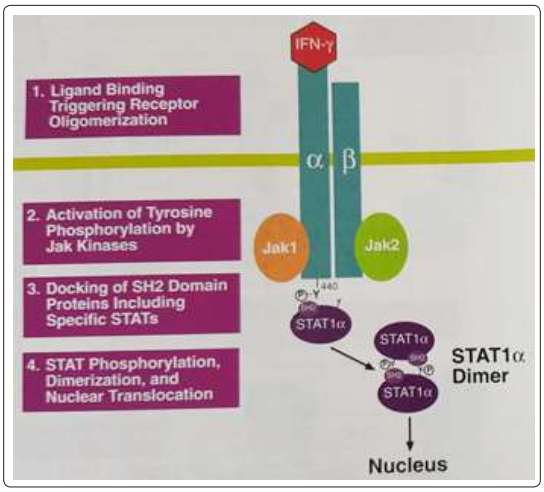
The cell surface receptors for IL1, TNF and epidermal growth factor (EGF) were rapidly clustered and internalized after exposure to UV radiation, leading to ligand-independent activation of both NFkB and the c-Jun N terminal kinase (JNKs) [1].



Jak STAT signal transduction pathways.

The Jak STAT pathways was first elucidated through careful analysis of signaling initiated by interferon receptors, but was subsequently shown to play a role in signalling by all cytokines that bind to members of the hematopoietin receptor family [1]. The Jak STAT pathway operated through the sequential action of the family of four nonreceptor tyrosinase kinases (the Jak or Janus kinases) and a series of latent cytosolic transcription factors known as STAT (signal transduccers and activators of transcription). The cytoplasmic portions of many cytokine receptor chain were noncovalently associated with one of the four Jaks (Jak1, Jak2, Jak3, Tyk2) [1].

Participationof Jak and STAT protein in IFNγ signaling was firstly acted by binding of human IFNγ (a dimer) to its receptor brought - about oligomerization of receptor complexes (composed of α and β chains). The nonreceptor protein tyrosinase kinase (Jak1 and Jak2) were activated and phosphorilated critical tyrosinase residues in the receptor such as the tyrosinase at position 460 of theα chain (γ 460). STATα molecules were recruited to the IFNγ receptor based on the afinity of their SH2 domains for the phosphopeptide sequence around γ460. Receptor associated STATα molecules than dimerized through reciprocal SH2 phosphotyrosine interactions. The resulting STATα dimers translocated to the nucleus and stimulated transcription of IFNγ regulated genes [1].



Cytokines modulated balans between humoral and cell based immune responses, and they regulated the maturation, growth and responsiveness of particular cell populations. Some cytokine enhanced or inhibited the action of other cytokine in complex ways [1].

Cytokine And Inflammasome Activation

IL1 was the common initiator of keratinocytes activation. Activated keratinocytes were hyperproliferative and produced paracrine signals to alert fibroblasts, endothelial cells, melanocytes and lymphocytes, as well as autocrine signals to adjacent keratinocytes, thus maintaining the activated state. The role of IL1 in the initiation of acne lessions was important. Comedones in vitro were rich in IL1α like bioactivity and IL1α immunoreactivity was shown in early phase (<6 hours) papules and uninvolved skin. In the uninvolved skin of acne patients, increased IL1α labelling was detected in all layers of both the interfollicular and down the follicle wall compared to control. In the epidermis of inflammed lessions, significant increased of IL1α labelling was also observed compared with skin but down the follicular wall increased IL1α labeling was only present in infundibullar basal cells. Furthermore, exposure to IL1α caused hypercornification of the infundibulum similar to that seen in comedones in isolated, cultured human infundibulum in vitro [10,12,21].

IL1α predominated in the epithelial cells including keratinocytes and IL1β appeared to be the dominant form of IL1 produced by monocytes, macrophages, langerhans cells and dendritic cells. IL1α was directtly biologically active, but IL1β need translated through caspase1 gene transcriptase to be actived.

Epithelial IL1α was stored in cytoplasm of the epithelial cells that comprised at interface with the external environment. Such cells when injured, could release biologically active 31 kDa IL1α

and therefore could initiated inflammation. Leukocytes, including dendritic and langerhans cells could release IL1β and unregulated released of IL1β caused significant tissue damage. The released of mature biologically active IL1β from cells was controled by several IL1β gene transcription (caspase 1 genes transcription that activated by transcription of the proteases). Than IL1 activated NFkB pathway that than produced more IL1 [1].

A molecules known as the IL1 receptor antagonist or IL1ra could bind to the ILIR1 and caused the quenching of IL1 mediated inflammatory responses [1].

IL18 was first identified by its capacity to induce IFNγ. IFNγ also known as IL1γ because of its homology to IL1α and IL1β.. Like IL1β it was translated as an inactive precursor molecule of 23kDa and was cleaved to active 18kDa species by caspase1. It was produced by multiple cell types in skin, including keratinocytes, langerhans and monocytes. IL18 induced proliferation, cytotoxicity and cytokine production by Th1 and NK cells, mostly synergistically with IL12. The receptor for IL18 was IL18R that was IL1R homologue, that was the part of innate imune system [1].

Released IL1 induced expression of endothelial adhesion molecules including E selectine, ICAM-1 and VCAM-1, as well as chemotactic and activating chemokines. This attracted monocytes, granulocytes and spesific population of memory T cells that bear Cutaneous lymphocytes Antigen (CLA) on their cell surfaces. If memory T cell encounter their antigen from the environment, their activation and subsequent cytokine production would amplify the inflammatory response [1].

Based on data obtained from in vitro studies, it had been hypothesized that the cytokine milieu simultaneously induced comedogenesis, and de-differentiation of sebocytes into a keratinocyte like phenotype and sebaceous gland atrophy. Pro inflammatory cytokines also induced adhession molecules on endothelial cells to facilitate recruitment of inflammatory cells into the skin. Subsequent to the proinflammatory stimulus by cytokines, expression levels of intercellular adhesion molecule 1 (ICAM1), E selectin and vascular cell adhesion molecule 1 (VCAM1) were increased in early stage inflammatory acne lessions [12].

Inflammasome were cytosolic protein complexes that recognized a diverse range of inflammation-inducing stimuli including both exogenous and endogenous signal e.g. microbial, stress and damage signals, and responded by activating caspase-1 and producing inflammatory cytokines IL1β and IL18. Recent reports supported Nod Like Receptor P3 (NLRP3) inflammasome activation in acne. Inflammasome protein NLRP3 and caspase1 were expressed in CD68+ macrophages and sebaceous glands in acne lessions in vitro and P Acnes triggered NLRP3 inflammasome activation in vitro resulting in IL1β released from sebocytes and monocytes cells but not from keratinocytes. Furthermore mature IL1β was expressed at the site of cutaneous inflammation, in the presence of macrophages surrounding the pilosebaceous units and in the sebaceous glands in acne lessions. P Acnes induced inflammasome activation depended on the internalization of the bacteria by antigen presenting cells, lysosomal maturation, activation of cathepsin B, generation of reactive oxygen species (ROS) and potasium efflux [10,21,22].

Analysis of skin biopsies from acne lessions using RT PCR, gene array and immunohystochemical staining techniques had demonstrated an increased expression of the inflammatory cytokines tumor necrosis factorα (TNFα), IL6, IL8, IL17A, interferonɣ (IFNɣ), IL21 and the anti inflammatory cytokine IL10. The concentration of IL17, IL4, IFNɣ and TNFα was elevated in serum samples of patients with moderate to severe nodulocystic acne [23].

In addition to cytokines IL1α and IL1β, P Acnes induced the production of pro inflammatory cytokines IL6, IL8, IL12, IL17A, TNFα, IFNɣ and granulocyte macrophage colony stimulating factor (GM-CSF) by human monocytes, keratinocytes or sebocytes in vitro [23].

TNFα was an important mediator of cutaneous inflammation and its expression was induced in the course of almost all inflammatory responses in the skin. Normal human keratinocytes (NHEK) and keratinocytes cell line produced substantial amount of TNFα after stimulation with LPS, UV light and irritant substance and contact sensitizers [1].

IL6 interacted with a hemopoitin receptor family member gp130 (glkycoprotein 130 kDa). IL6 was the important cytokine for the skin and was subject to dysregulation in several human skin manifestation diseases. IL6 was produced in a regulated fashion by keratinocytes, fibroblast and vascular endothelial cells, as well as by leukocytes infiltrating the skin. IL6 stimulated the proliferation of human keratinocytes under some condition, and the expression was increased in psoriasis and acne vulgaris [1].

IL12 had active form a heterodimer of two protein p35 and p40. IL12 was produced by Antigen presenting cells as dendritic cells, monocytes and macrophages as well as certain B cells in response to bacterial component, GM-CSF and IFNγ. Activated keratinocytes was the additional source of IL12 in skin. Human keratinocytes constitutively made the p35 subunit and the expression of p40 subunit could be induced by stimuli such as contact allergens, phorbol esthers and UV radiation.

IL12 was immunoregulatory cytokine for initiation and maintenance Th1 response. Th1 responses was depend on IL12, provided protective immunity to intracellular bacterial pathogen and mediated Th1 response in autoimmune disease. IL12 had simulatory effects on NK cells, promoting their proliferation, cytotoxic function, and the production of cytokine including IFNγ and stimulated protective antitumor immunity [1].

So IL12 activated innate (stimulated proliferation and cytotoxicity of NK cells) and adaptive (stimulated Th1 response) immune response.

IL10 was one of several cytokine that primarily exert regulatory rather than stimulatory effects on immune response. The ligand binding chain of the receptor was homolog to the receptor for IFN α,β,ɣ and signaling events mediated through the IL10 receptor use Jak STAT pathway [1].

IL10 was produced by Th2 T cells that inhibited cytokine production after activation of T cells by antigens and APC. IL10 acted through the surface receptor on macrophages, dendritic cells, neutrophyls, B cells, T cells and NK cells.

Inhibitory effect of IL10 on APC such as monocytes, macrophages

and dendritic cells included inhibition of expression of class II MHC and costimulatory molecules (e.g. B7-1, B7-2) and decreased production of T cell stimulating cytokines (e.g. IL1, IL6 and IL12). But IL10 stimulated (rather than inhibited) B cell synthesis of class II MHC molecules and costimulates the growth of mast cells [1].

Epidermal keratinocytes was the major source of IL10 in skin. IL10 production by keratinocytes was upregulated after cell activation and one of the stimuli for activation was UV irradiation. UV induced keratinocytes production lead to local and systemic immunity. Immunosupresive effects that occured after exposure to UV light exposure were the result of the liberation of keratinocytes derived IL10 into the systemic circulation. The absence of IL10 exaggerated irritant and contact sensitivity response [1].

Pro Inflammatory Cytokine In Acne Vulgaris Inflammation

It was reported that released IL1α by keratinocytes of the sebaceous duct was pivotal in the life cycle of the comedo, mediating both its development and its spontaneous resolution [18].

The dermal inflammation was not been caused by bacteria in the dermis. It was probably resulted from biologically active mediators (IL 1α, IL β, TNFα,IL2, IL6, IL8, IL10, IL12,IL17, IL 18, IFNɣ) that produced in innate and adaptive immune system, that diffused from the follicle where they were produced by the binding of PAMPs/ Pathogen Associated Molecular Patterns bacterials to TLR2 receptor (and TLR4 receptor) of monocytes, sebocytes, keratinocytes, perifollicular and peribulbar macrophages, langerhans cells and other immune cells, activation of intra cytoplasm NLRP1 and 3, activation of PAR2 genes and through CD4+ of sensitized T lymphocytes [4,6,23,28].

There was an ability of innate immune system to use TLR2 receptor to recognonized microbial pattern and initiated immune response in cutaneous disease [13]. In innate immune system bacteria also activated intra cytoplasm NLRP3 caused pyroptosis and activated PAR2 gene that produced cytokine caused skin inflammation, tissue destruction and scar formation, that was initiated by protease enzyme that was released from P acnes.

TLR 2 receptor induced inflammatory response and the development of antigen spesific adaptive immunity [6].

Pro inflammatory cytokine (mainly IL1α) stimulated abnormal keratinocyte proliferation, differentiation and hypercornification that revealed obstructions, than there were follicles impaction and distention that formed comedos [2,3,13]. As the retained cells blocked the follicular opening, the lower portion of the follicle was dilated by entrapped sebum. Disruption of the follicular epithelium permitted the discharged of the follicular dermis [2]. The combination of keratin, sebum and microorganism led to pro inflammatory mediators releasing and lymphocytes, neutrophyls and foreign body giant cells accumulating [2]. In the early inflammation, inflammation was due to pro inflammatory mediators that moved through the duct wall into the dermis, and had not been caused by the duct ruptured [3]. Interleukine 1α that mainly produced by keratinocytes, was a dominant proinflamatory cytokine that played role in comedonal acne vulgaris inflammation process [4]. Other pro inflammatory cytokines that were produced were IL1, IL2, IL6, IL8, IL12, IL4, IL10, IL17, IL18, TNFα, IFNɣ [4,10,18,25].

Some kind of pro inflammatory cytokines that were produced by innate and adaptive immunity in acne vulgaris in some journals were :

- ✓ TLR 2 receptor of the monocytes bound to *P acnes* to produce IL12, IL8 pro inflammatory cytokine [24].
- ✓ NLRP3 inflammasome of the human sebocytes and monocytes as mediated pathway bound to live *P acnes* in the sebaceous glands through caspase 1 expression & activation to produce IL1β [22,26].
- ✓ TLR 2 receptor of the keratinocytes bound to *P acnes* to produce IL8, Human β Defensin 2 pro inflammatory cytokine [24].
- ✓ TLR2 receptor of human keratinocytes bound to *P acnes* through PAMPs-Pathogen Associated Mollecular Paterns to produce IL1α in 7 days of exposures that induced comedogenesis.27 PAMPs were such as peptidiglycan (PG) and lipopolysacharida (LP) of P acnes [6].
- ✓ PAR 2 of the keratinocytes bound to *P acnes* to produce IL 1α, IL8, TNFα [24].
- ✓ TLR2 receptor of human monocytes and skin surface macrophages in human pilosebceous bound to microbial agent (*P acnes*, gram positive coccus) through NFkB/Nuclear Factor kappa light chain transcription factor enhancer of activated B cells activation [6,17] and MAPK (Mitogen Activated protein Kinase) cascade [6] to sinthesize and release of IL1, IL2, IL6, IL10,IL12, IL8. TNFα, IL1β [17] mediated pathogen destruction via effector cells [18].
- ✓ TLR 2 receptor of monocytes bound to gram positive coccus to produce IL12.28
- ✓ Monocytes bound to *P ovale* (lived or heat killed, opsonized P ovale through alternative complement activation pathway more stimulated than non opsonized) to produce IL8, IL1α [28].
- ✓ Monocytes bound to gram positive bacteria to produce TNFα, IL6 [28].
- ✓ Monocytes bound to Gram negative bacteria bound to produce TNFα. IL1. IL6 [28].
- ✓ Human keratinocytes binding P.acnes produced IL18, like monocytes and macrophages do, being two major sources for this molecule. IL18 (that innitialy described as IFNɣ inducing factor) acted directly to Natural killer cells to stimulate IFNɣ synthesis and upregulated their killing capacity [18,25]. It was believe that IL18 derived from keratinocytes might be than involved in cutaneous Th1 type adaptive immune response [25].
- ✓ P. Acnes binding CD4+ T cells and neutrophyls, several other types of immunological cells including Tc cells, group 3 innate lymphoid (ILC3) cells, gama teta cells, NK cells, Mast cells and macrophages produced IL17 [29].

IL 8 induced chemotactic factors might played an important role in attracted neutrophyl to the pilosebaceous unit that led to release lysosomal enzime, then led to rupture follicular epithelium and further inflammation.. Furthermore *P acnes* released lipases, proteases, hyaluronidase which contributed to tissue injury. IL 8 induced chemotaxis and activation of neutrophyl and T cells [18,28].

IL12 promoted development of Th1 mediated immune response. And overproduction of Th1 cytokine such as IL 12 was implicated in the development of tissue injury in a certain autoimmune and inflammatory disease [18].

IL1α was low activated by lymphocytes, chemotaxis, activation of neutrophyl than induced inflammation [28]. Therefore interaction of *P ovale* and phagocytic cells might serve to amplify the inflammatory response and encourage further recruitment of phagocytic cells. *P ovale* upregulated phagocytic cells (macrophages) thus provided enhancer protection to bacterial and tumor cells. There were down regulated of pro inflammatory cytokine by removed lipid. Langerhans/macrophages were able to take up antigen (acted as antigen presenting cells/APC) and then were presented to T cells [28].

P acnes activated monocytes cytokine relased through the pattern recognition receptors (PPRs), for example TLR2 receptor of the innate immune system [18]. So TLR 2 receptor was PPRs. TLR2 receptor activation contributed to the pathogenesis of acne, suggesting that these cells promoted inflammatory responses at the site of the disease activity and induced pro inflammatory cytokine production. Release of pro inflammatory cytokines that were mediated through TLR2 receptor had harmful effect in acne by promoting inflammation and tissue destruction [18]. So TLR 2 receptor was a logical target for therapeutic intervention to block inflammatory cytokine response in acne and other inflammation condition which tissue injury was detrimental to the host [18,27]. Isotretinoin down regulated TLR2 that induced cytokine response [6].

Infestation of the organism itself was not the main caused of the disease but was rather caused by the various inflammatory responses that were initiated by microbial agents that led to destruction of the host tissue. Such responses were : the formation of immune complex, the recruitment and activation of neutrophyl and monocytes, the released of cytokines, released of dependent enzymes [18].

Chemokines

Chemokines were a large superfamily of small cytokines that had two major functions. First, they guided leukocytes via chemotactic gradient in tissue. Second, a subsets of chemokines had the capacity to increase the binding of leucocytes via their integrin to ligands at the endothelial cell surface, thus facilitating firm adhesion and extravasation of leucocytes in tissue [1].

Chemokines were small proteins with mollecular weights in the 8-10kDa range. They were synthesized constitutively in some cells and could be induced in many cell types by cytokines. Innitially associated only with recruitment of leukocyte subsets to different inflammatory sites, it had become quitely clear that chemokines played roles in angiogenesis, neural development, cancer metastases, hematopoiesis and infectious diseases. Chemokines and their receptors were accepted as vital mediators of cellular trafficking [1].

Molecular signalling patways were initiated by the binding of chemokines to its cognitive receptors. Expression pattern of chemokine receptors were noted, particularly in light of the many cell types of immune cells that potentially could be recruited to skin under inflammatory conditions. Individual chemokine receptors were highlighted in regard to observed biologic function, including facilitation of migration of effector T cells into the skin and the egress to antigen presenting cells on the skin. There were some role of chemokines and chemokines receptors in disease - atopic dermatitis, psoriasis, cancer and infectious disease [1].

Chemokines could be grouped into four subfamilies based on the spacing of amino acid between the first two cystein. The CXC chemokines (also called α chemokines) showed C-X-C motif with one non conserved amino acid between the two cysteines. The other major subfamily was termed the CC subfamily (or β chemokines) because the lack of the additional amino acid. The two remaining subfamilies contained only one member each of the C subfamily was represented by lymphotactin and fractalkine was the only member of the CXXXC or CX3C [1].

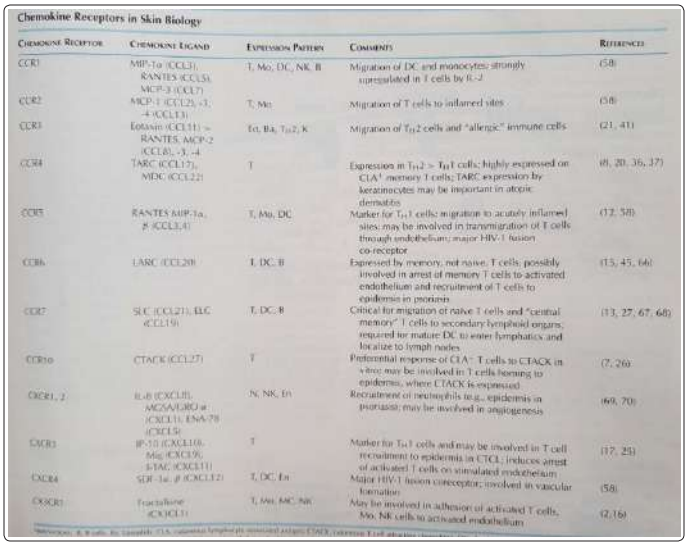
Chemokines could also be assigned to one of the two broad that perhaps overlapping functional groups. One type mediated the attraction and recruitment of immune cells to the site of active inflammation (e.g regulated of activation normal T cells expressed and secreted/RANTES, macrophage inflammation protein/MIP/1α/β, and Liver activation regulated chemokines/LARC. While other chemokines appeared to play roles in constitutive and homeostatic migration pathways (e.g. Secondary lymphoid tissue chemokines/ SLC and stromal derived factor 1/SDF1) [1].

Because of the complexity and redundancy in nomenclature of chemokines, Ziotbic and yoshie proposed for systemic nomenclature for chemokines based on the type of chemokines (C, CXC, CX3C or CC) and a number based on order discovery. For example, SDF1, a CXC chemokines had the systemic name CXCL12 [1].

Most chemokines had a net positive charges and these proteins tend to bind to negatively charged of carbohydrates presented on GAGs/glycosaminoglycans or peptidoglycans. The ability of positively charged chemokines to bind to GAGs was through to enable chemokines to preferrentially associated with the lumenal surface of blood vessels despite the presence of shear forces from the blood that would otherwise washed the chemokines away [1].

IL8 was a chemokines that played role in acne inflammation process. IL 8 induced chemotactic factors might played an important role in attracted neutrophyl to the pilosebaceous unit that led to release lysosomal enzime that led to rupture follicular epithelium and further inflammation [17]. Furthermore *P acnes* released lipases, proteases, hyaluronidase which contributed to tissue injury [17]. IL 8 induced chemotaxis and activation of neutrophyl and T cells [18].

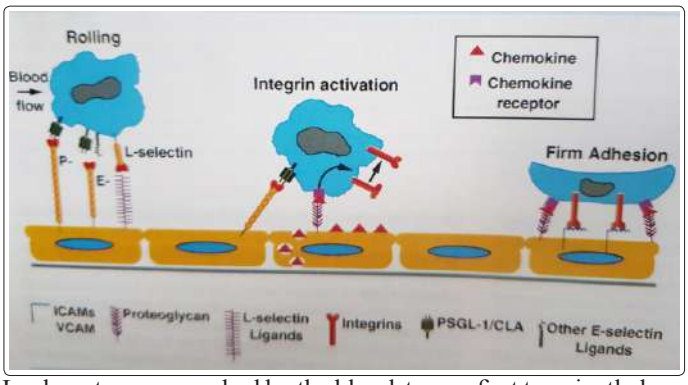
Chemokine Receptors In Skin Biology



Chemokine Receptors in Skin Biology

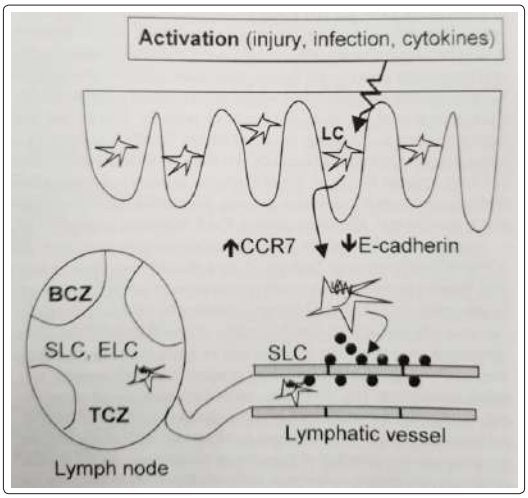
Chemokine Receptor	Chemokine Ligand	Expression Pattern	Comments	References
CCR1	MIP-1α (CCL3), RANTES (CCL5), MCP-3 (CCL7)	T, Mo, DC, NK, B	Migration of DC and monocytes; strongly upregulated in T cells by IL-2	(58)
CCR2	MCP-1 (CCL2), -3, -4 (CCL13)	T, Mo	Migration of T cells to inflamed sites	(58)
CCR3	Eotaxin (CCL11) > RANTES, MCP-2 (CCL8), -3, -4	Eo, Ba, $T_{H}2$, K	Migration of $T_{H}2$ cells and "allergic" immune cells	(21, 41)
CCR4	TARC (CCL17), MDC (CCL22)	T	Expression in $T_{H}2 > T_{H}1$ cells; highly expressed on CLA^{+} memory T cells; TARC expression by keratinocytes may be important in atopic dermatitis	(6, 20, 36, 37)
CCR5	RANTES, MIP-1α, β (CCL3,4)	T, Mo, DC	Marker for $T_{H}1$ cells; migration to acutely inflamed sites may be involved in transmigration of T cells through endothelium; major HIV-1 fusion co-receptor	(12, 58)
CCR6	LARC (CCL20)	T, DC, B	Expressed by memory, not naive, T cells; possibly involved in arrest of memory T cells to activated endothelium and recruitment of T cells to epidermis in psoriasis	(15, 45, 66)
CCR7	SLC (CCL21), ELC (CCL19)	T, DC, B	Critical for migration of naive T cells and "central memory" T cells to secondary lymphoid organs; required for mature DC to enter lymphatics and localize to lymph nodes	(13, 27, 67, 68)
CCR10	CTACK (CCL27)	T	Preferential response of CLA^{+} T cells to CTACK in vitro may be involved in T cells homing to epidermis, where CTACK is expressed	(7, 26)
CXCR1, 2	IL-8 (CXCL8), MGSA/GRO-α (CXCL1), ENA-78 (CXCL5)	N, NK, En	Recruitment of neutrophils (e.g., epidermis in psoriasis); may be involved in angiogenesis	(69, 70)
CXCR3	IP-10 (CXCL10), Mig (CXCL9), I-TAC (CXCL11)	T	Marker for $T_{H}1$ cells and may be involved in T cell recruitment to epidermis in CTCL; induces arrest of activated T cells on stimulated endothelium	(17, 25)
CXCR4	SDF-1α, β (CXCL12)	T, DC, En	Major HIV-1 fusion coreceptor; involved in vascular formation	(58)
CX3CR1	Fractalkine (CX3CL1)	T, Mo, MC, NK	May be involved in adhesion of activated T cells, Mo, NK cells to activated endothelium	(2,16)

multistep model of leukocyte recruitment in bloodstream [1]



Leukocytes were pushed by the bloodstream, first transiently bound or rolled on the surface of activated endhothelial cells via rapid interaction with P-, E- or L-selectin. Chemokines were secreted by endothelial cells and bound to peptidoglycans that presented chemokine molecules to chemokine receptors on the surface of leukocytes. After chemokines ligation, intracelluler signaling events led to a charged in the conformation of integrins and charged in their distribution on the plasma membrane resulting in 'integrin activation'. These charged resulted in high affinity/avidity binding of integrin endothelial cells intercellular adhesion molecules/ICAMs and vascular cell adhesion molecules/VCAM-1 in a step termed firm adhesion, which was then followed by transmigration of leukocytes between endothelial cells and into tissue.

Chemokines in the trafficking of Dendritic Cells from skin to regional lymph Nodes [1]



Langerhans cells were activated by a variety of stimuli including injury, infectious agents and cytokines such as IL1β and TNFα. Having sample antigens, the activated LC downregulated E cadherin and strongly upregulated CCR7. Sensing the CCR7 ligand and SLC, that were produced by lymphatic endothelial cells, the LC migrated into lymphatic vessels, pas sively flowed to the lymph nodes and stop in the T cells zone/TCZ that were rich in two CCR7 ligand-SLC.

Chemokine mediated migration of T cells.

Antigen inexperienced T cells were termed naive and could be identified by the expression of three cell suface proteins. CD45RA (an isoform of the pan leukocyte marker), L-selectin and the chemokine receptor CCR7. These T cells migrated efficiently to secondary lymphoid organ (e.g. Lymph nodes), where they might make contact with antigen bearing DC from periphery. Once activated by DC presenting antigen, T cells then expressed CD45RO, were termed memory T cells, and appear to express a variety of adhesion molecules and chemokines receptors that facilitated their extravasation from blood vessels to inflammed peripheral tissue. A spesific subset of CCR7 negative and L-selectin negative memory T cells had been proposed to represent an effector memory T cells subset that was ready for rapid development at peripheral sites termed of cytotoxic activity and ability to mobilize cytokines [1].

Although chemokines were both secreted and soluble, the net positive charge on most chemokines enabled them to bind to the negatively charged proteoglycans such as heparan suphate that were presented on the lumenal surface of endothelial cells. Thus, chemokines were presented to T cells as they rolled along the lumenal surface. After ligand binding, chemokine receptors sent intracellular signal that led to increases in the affinity and avidity of T cells integrins such as LFA-1 and very late antigen-4 (VLA-4) for their endothelial receptors ICAM-1 and vascular adhesion molecules-1 (VCAM-1). Only a few chemokine receptors (CXCR4, CCR7, CCR4 and CCR6) were expressed in the sufficient levels on resting peripheral blood T cells to mediate this transition. With activation and interleukine IL2 stimulation, increased number of chemokine receptors (e.g. CXCR3) were expressed on activated T cells, making them more likely to respond to other chemokines. In several different systems, inhibition of spesific chemokines produced by endothelial cells dramatically influenced T cell arrest *in vivo* and *in vitro* [1].

Leukotrien (Eicosanoids)

Arachidonic acid and its metabolites termed eicosanoids were ubiquitous bioactive mediators formed from unsaturated fatty acids presented in cell membranes. Eicosanoids function as local hormones, regulating many important physiologic and pathologic processes such as pain, fever, clotting and parturition. Medication that altered their formation were readily available. Biochemically they were formed by the addition of oxygen to polyunsaturated fatty acids such as arachidonic acid and linoleic acid, which led to the formation of large array of biologically active mediators [1].

Eicosanoids had potent biologic effect in picomolar amounts. The extreme biologic potency of this class of compounds necessitates careful regulation of their synthesis and degradation. Arachidonic acid was stored in the phospholipids of cell membranes and was released by phospholipases after the spesific stimulation of cell surface receptors.They was careful regulated released by receptor stimulation, where arachidonic acid was released during membrane dissolution induced by cellular necrosis or apoptosis. Once cleaved from the membrane, arachidonic acid was immediately oxygenated to form products that were released from the cell or were esterified into membrane phospholipids. Because arachidonic acid metabolits were not stored, the synthesis of products of arachidonic acid was synonymous with the release [1].

Eicosanoid synthesis had spesific structural requirement. Eicosanoids were derived from essential omega 6 fatty acid (eicosatetraenoic acid-20:4n-6 and eicosatrienoic acid -20: 3n-9) and their omega 3 derivates, eicopentaenoic acid (EPA, 20: 5n-3) and docosahexaenoic acid (DHA, 22: 6n-3). Among these fatty acids, arachidonic acid. also known as 5,8,11,14 eicosatetraenoic acid (20: 4n-6) was ussual substrate for eicosanoic formation and was ussualy stored in the sn-2 position of phospholipid [1].

Arachidonic acid and its metabolits acted locally to regulate autocrine and paracrine functions. Their metabolism did not affect the function of cells at distant sites. These local action were regulated in several ways. The array of product that could be synthesized from arachidonic acid was not produced in each cell type, each cell had a unique set of enzymes and receptors that permitted rapid, selective, local regulation. Each cells had characteristic set of enzymes for eicosanoid synthesis, which determined its product profile. The nature of stimulus also influenced the products that were formed by each type of cel, e.g. Particulate versus soluble [1].

In addition of regulating the synthesis of this compounds, cells also tune their response by regulating the expression of their receptors for detecting prostaglandins and leukotrienes. A cells in its resting state might express only a few products, but after cytokine stimulation it might synthesize new enzyme and receptors or might downregulate receptors selectively [1].

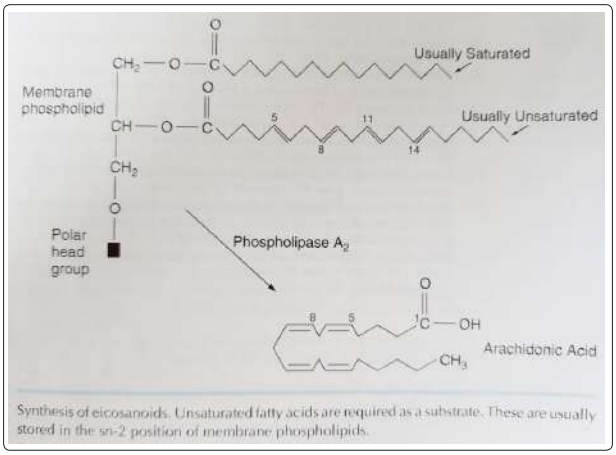
Because arachidonic acid was a membrane constituent, its released from membrane phospholipids by phospholipase (an enzyme) was a key regulatory event in initiating eicosanoid synthesis. Phospholipases had unique substrate interaction characteristics that influenced how they could act to release substrate. Because the substrate was present in membrane (an equilibrium reaction), after which its scoots on the membrane surface to liberate its membrane [1].

Multiple phospholipids existed that could selectively hydrolized fatty acid from phospholipid was presented in cell membrane. They were named for the location of the bond they attacked during substrate hydrolysis. The enzymes attacking these four sites of hydrolysis were named phospholipase A1 (PLA1), phospholipase A2 (PLA2), phospholipase C (PLC) and phospholipase D (PLD). Mammalian PLA2 were divided into two major types, secretory and cytosolic [1].

Secretory PLA2. It required calsium for catalysis, had low molecular weight (14kDa), had 2 groups pancreatic/digestive type I and non pancreatic/inflammatory type II. I played role in mast cell mediated inflammation. It was thought to be key in the release of arachidonic acid in inflammatory disease state, such as arthritis.

Cytosolic PLA2. Some of it was calcium dependent, had high molecular weight (39-100 kDa). It thought to be responsible for the majority of eicosanoid product formation and as a major source of arachidonic acid in skin.

PLC. In some tissue such as platelets, arachidonic was released indirectly by PLC. Once arachidonic was in the cytosol, there were three main pathways of metabolism, cyclooxygenase, lipooxygenase and monooxygenase. Cyclooxygenase metabolisms led to the synthesis of prostaglandins and tromboxanes, which interacted with spesific receptors to initiated tissue responses. Cyclooxygenase had two isoform : COX1 and COX2 located in the endoplasmic reticulum- its exp[ression was inducible by growth factor and IL1. Lypooxygenase enzymes catalized the formation of 5-, 12-, 15- hydroxyperoxyeicosatetraenoic acid/HPETE intermediated, which were subsequent reduced. Leading to the formation of HETE. 5 Lypooxygenase was important produced leukotriene from arachidonic acid. Monooxygenase (member of cytochrome P450 family) were responsible in the formation of arachidonic acid product. That important for eicosanoic degradation and in the formation of product with biologic activity [1].



Synthesis of eicosanoids. Unsaturated fatty acids are required as a substrate. These are usually stored in the sn-2 position of membrane phospholipids.

Prostaglandin

The E series of prostaglandine were important modulator of normal cell proliferation and played an important role in wound repir. Production of collagen fibrils in the dermal matrix was also decreased in the absence of PGE2 [1].

Prostaglandin D2 (PGD2) was the primary prostaglandin that was synthesized in mast cells as an important mediators in allergic responses. PGD2 was the predominant prostaglandin formed by Langerhans cells. PGD2 was found abudantly in central nervous system and male genitalia. Two types of enzymes synthesize PGD2, lipocalin/brain type and hematopoietic type that was predominant in the skin. In the skin PGD2 acted as peripheral vasodilator, allergic and inflammtory mediator, mediator of nociceptive responses and an inhibitor of platelet aggregation. Its effect outside the skin included sleep induction an centrally induced hypothermia [1].

In small vessels of the skin, TXA2, PGE2, and PGI2 were key mediators in regulating peripheral vascular tone and clotting. Bacteria stimulated platelet, monocytes and macrophages in the blood element, synthesized TXA2 through the action of microsomal P450 enzynes - tromboxane synthetase. TXA2 was potentn inducer of vasoconstriction and platelet aggregation, had very short half life (30 sec) that allowed modulation of vasoconstriction, it spontaneously hydrolized to TXB2- an inactive degradation product [1].

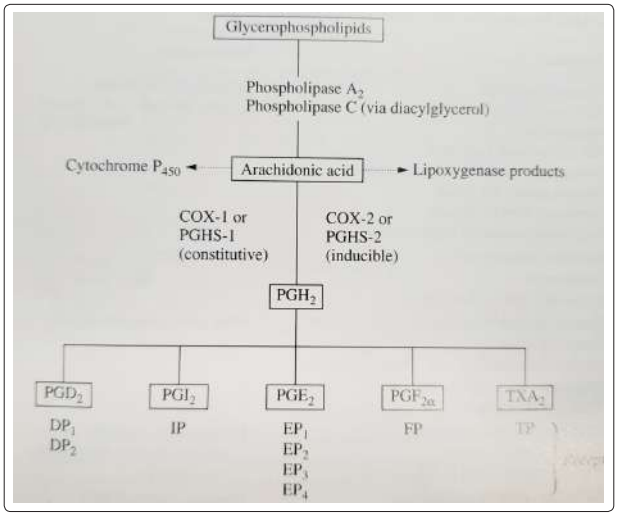
To blance the formation of potent vasoconstrictor TXA2by blood element, vascular endothelium and smooth mucle actively synthesied PGI2 and PGE2, which were potent vasodilators via microsomal cytochrome P450 synthetase. PGI2 was an antiaggregatory agent that capable to stop the proliferation of platelet thrombus, had short 10 minutes half life, spontaneosly hydrolozed to PGF1 alfa. PGE2 was synthetized in rthe endothelium, caused capillary smooth muscle dilatation and plasma exudation, potentiated histamin and bradikinin that inducedc effect of vascular permeability and enhanced the sensation of pain. PGE2 had more long lasting half life than PGI2.

The balance of synthesized of vasodilator in vascular cells and vasoconstrictor released from blood cells determined vascular tone and inflammatory pain [1].

PGF2alfa was synthesized by keratinocytes in the skin, by the action of PGF synthase. PGF2 alfa was a vasopressor, stimulated fibroblast proliferation, and it was important in labor delivery. PGD2 could

also be metabolized to a diastereoisomer of PGF2 alfa by NADPH dependent mechanism [1].

Prostaglandine produced their effects through interaction with spesific G protein linked receptors. Prostaglandin receptor had structural motif common to G protein coupled rhodopsin type receptors with seven putative trans membrane domain. Spesific receptor had been identified for each major prostaglandine types, receptors TXA2 was TP, receptor of PGF2alfa was FP, receptors of PGE2 were EP1, EP2, EP3, EP4, receptor of PGI2 was IP and two distinct receptors of PGD2 were DP1 and DP2. Stimulation of the prostaglandine receptors might trigger a rise in calcium, mediated the rise in intracellular c AMP or inhibited c AMP formation that induced smooth mucle contraction and relaxation and mediated Ca2+ mobilization [1].



Leukotrien / LTB4

Leukotrienes were synthesized predominantly by 5-lipooxygenase enzymes that were found in leucocytes, macrophages, mast cells and lung epithelium, that metabolized arachidonic acid to form leukotriene intermediate (LTA4) that was hydroxylazed by LTA4 hydrolase (a cytosolic enzyme that was found in leukocyte, lung, heart and intestine) to form LTB4 and LTC4. Leukotrienes had individual export carier system, that was ATP dependent, than leukotriene released from the cells [1].

5 lipooxygenase also found in keratinocytes. There were three classes of lipooxygenase enzymes that insert oxygen into -5, -12, -15 carbon of arachidonic acid. 5 lipooxygenase translocated to the nuclear membrane in the presence of Ca2+ [1].

LTB4 was predominant eicosanoid metabolit of neutrophyls that also produced by macrophages. LTB4 was most potent chemotactic and chemokinetic substance, could induce degranulation of polymorphonuclear leukocytes and their adherence to vascular endothelium. LTC4 (a cysteinyl leukotrienes) was known as slow reacting substance of anaphylaxis. The removal of glutamic acid from LTC4 by gama glutamyl transpeptidase produced LTD4 [1].

Leukotriene receptors were expressed in the airway as well as in the smooth muscle, microvasculature, blood elements and skin. LTB4 receptors were linked via G protein to PLC. LTC4 also appeared to be coupled via a Gq protein into IP3 turnover and increased in intracellular Ca2+ [1].

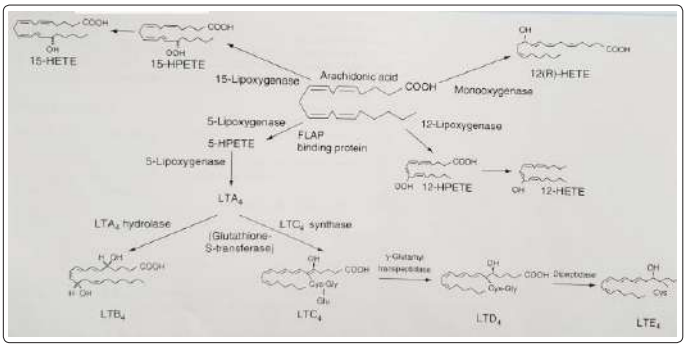
To eliminate LTB4 and LTC4, oxidative inactivation occured in combination of hydrogen peroxide produced through the respiratory burst of activated cells and extracellular chloride ion to produce hypocloric acid, which attacked the sulfur of the cysteinyl adduct. Produced compound that capable of forming DNA adducts, increasing epidermal growth factor-dependent signaling and stimulating progression of breast carcinoma [1].

Arachidonic 12 lipooxygenase activity was found in the platelets, leukocytes, mast cells and epithelial cells. 12-S-hydroxyeicosatetraeioic acid reported to enhance tumor cell growth, endhotelial cell invasion and α11bβ 3 integrin expression [1].

15 lip[ooxygenase metabolic pathway was predominant in the eosinophyls and human airway epithelial cells. 15 HETE induced hyperalgesia. It could also influence the synthesis of lipooxygenase and leukotriene product in mast cells, platelets, neutrophyls and eosinophyls that implicated in atherogenesis [1].

Lipoxin were the family compounds that could be derived from arachidonic acid by serial action of -5,-12,-15 lipooxygenase, followed by further lipooxygenase metabolism. Lipoxin A4 caused arteriolar dilatation, antagonized LTB4-induced inflammation, and inhibits polymorphonuclear leukocytes adhesion to endothelial cells. Lipoxin B4 stimulated monocyte colony formation and both products were bronchoconstrictors. Seven transmembrane domain type receptor of lipoxin and its mRNA were found in the lung, myeloid cell types and tissues with high degrees of phagocytes infiltrates. Lipoxin A4 binding on PMN was a G protein coupled. Lipoxin also acted with LTC4 and LTD4 receptor, thus their actions might be like those leukotrienes at the receptor site [1].

Cytochrome P 450 enzymes could also metabolized eicosanoids. Epoxy acid were able to mobilize microsomal calsium and to alter K+ and Na+ ion flux, regulated ion transfer that made monooxygenase products important in regulating water balance, that was also predominant metabolic pathway for LTB4 [1].



Complement

Complement was a key component of innate immunity but its role in the pathogenesis of acne was not very well understood. In addition to its important role in cutaneous defence against microbial infection, complement also medited inflammation and tissue injury [30].

Complement was a system of plasma proteins that could be activated directly by patogens or indirectly by pathogen binding antibody, leading to a cascade of reactions, that occured on the surface of pathogens and generated active comnponents with various effector function. Complement was a system in the blood of 12 or more

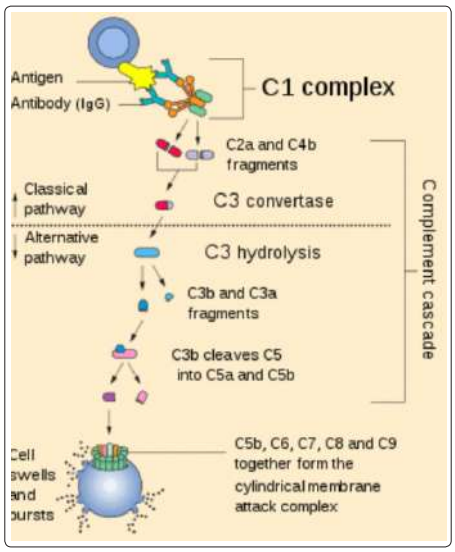
proteins that reacted a cascade to a cell displaying immune complexes or foreign surfaces, acting in various combinations to coat the cells and promote phagocytosis, makes hole in the cell wall or enhance the inflammatory response. The complement system was a part of the innate immune system that enhanced the ability of antibodies and phagocytic cells to clear microbes and damaged cells from organism, promoted inflammation and attacked the pathogens cell membrane [1].

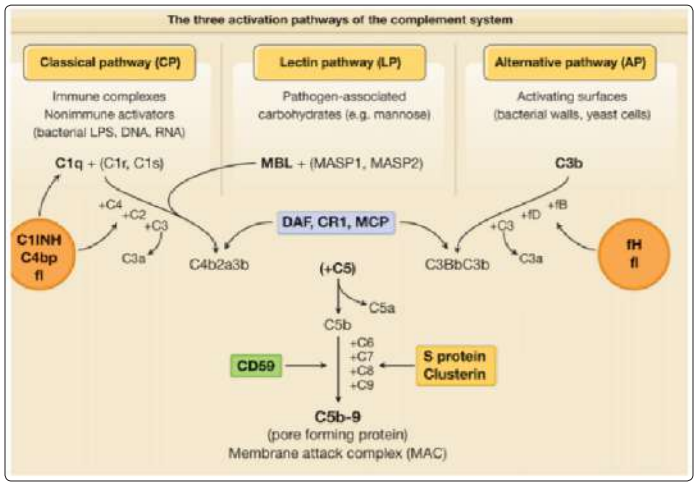
There were 2 complement activation : classical pathway (that was mediated by immune complex) and altenative pathway (that was mediated by yeast or bacterial cells) [30]. There was complement activation that involved in the early to later stages of inflammation and *P acnes* were capable for triggering both the alternative and classical complement pathways [1,3,4,28].

Comedonal contents and P acnes had been shown to activate complement via both classic and alternative pathways. Complement-3 (C3) immunoreactivity had been detected in early stage inflammatory in acne lessions in the walls of small dermal blood vessels and the dermo epidermal junction. In the late stage inflammatory lessions C3 deposition was much less prominent [10].

Complement activation caused lysis bacteria and virus, opsonization, inflammation [28].
P ovale (through an alternative pathway of complement activation) activated cellular immune response and humoral immune response [28]. Complement was the part of immune system that enhanced the ability of antibodies and phagocytic cells to clear microbes and damage cells from an organism, promoted inflammation and attacked the pathogen plasma membrane. It was part of innate immune system (which was not adaptable and did not change over the course of individual`s lifetime) and it could been recruitted by the adaptive immune system to finish the action [3,4].

P acnes antigens activated the complement system and provided migration of free radicals, neutrophyls and macrophages with the following production of proteolytic enzymes, IL1α. IL 1β, IL8 and TNFɣ, which caused inflammation and complex cascade of pathogenetic mechanisms of the disease. The role of cellular and humoral immunity and cytokine activity in acne was still a subject of study [18].





Toll Like Receptors And Nod Like Receptors

During an infection, one of the first form of defense employed by the innate immune response was a group of Pattern Recognition Receptors (PPRs) encoded in the germline to recognize mollecular pattern expressed by invading pathogens. In response to P acnes, these might either be on the membrane surface of the innate immune cells (Toll Like Receptors/TLRs and C Type Lectin Receptors/CLRs) or inside the cytoplasm (Nod like Receptor/NLR and RIG I Like Receptors/RLRs) [31,33].

NOD Like Receptors

NLRP3 GENE [22,26,32]

Nucleotide Oligomerization Domain (NOD) Like Receptors (NLRs) were an important class of cytosolic pattern recognition Receptor for microbial molecules and danger signals and triggering inflammation and anti microbial response. NLRs were part of inflammasome complexes, which were a central component for regulation IL1β maturation and secretion. Involvement of inflammasome NLRP3 complexes (NLRP3 signaling) were inducing inflammatory responses in skin diseases, including Psoriasis, extracellular Staphylococcal infection and P acnes infections espesially in human monocytes [22].

Inflammasome was a multiprotein oligomer consisting of caspase 1, PYCARD, NALP, and sometimes Caspase5 (also known as caspase 11 or ICH3). It was express in myeloid cells and was a component of innate immune system. The inflammasome promoted the maturation of inflammatory cytokines IL1β and IL18. NLRP3 inflammatory pathway did not need TLRs. Inflammasome could be detected in cytoplasmic DNA that might be threatening and strengthen their innate response. Inflammasome had been shown to induce cel pyroptosis, a programme cell death distinct from apoptosis.

Human monocytes responded to P Acnes and secreted mature IL1β partially via NLRP3 mediated pathway. When monocytes were stimulated by live P Acnes, caspase 1 was upregulated induced IL1β secretion in NLRP1 and NLRP3 mediated pathway, was an innate immune response, that caused inflammation in acne pathogenesis [22].

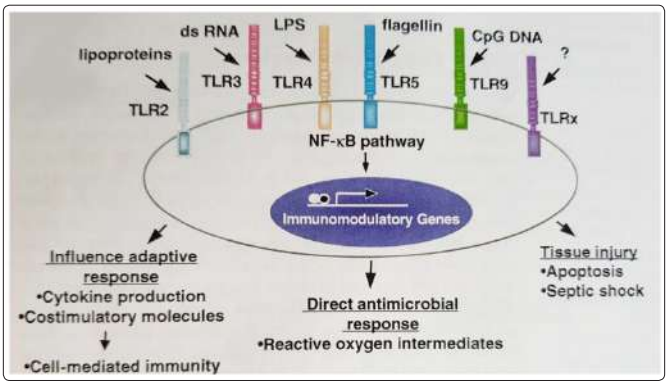
Mature ILβ required proteolitic cleavaged by inflammatory caspase 1. P Acne induced the m RNA expression of both caspase 1 and caspase 5, but only caspase 1 was required for IL1β secretion. Potasium eflux was required for P Acnes induced IL1β secretion.

NLRP3 activity had been shown to be induced or increased by low intracellular potasium concentrations, virus, bacteria, bacterial toxin liposome, cholesterol crystal, in organic particle (titanium dioxide, silicon dioxide, asbestos). NLRP3 activation was involved in sleep regulation [22].

The activation of NLRP3 inflammasome by P Acnes was dependent on protease activity and reactive oxygen species generation/ P Acnes induced activation of NLRP3 inflammasome, activated caspase 1 and induced IL1β secretion [26]. IL1β had been shown as a potent inducer of pro inflammatory cytokines IL6, IL8 in sebocytes suggesting the potential role in diseases of pilosebaceous unit such as acne [22].

Inflammasome were cytosolic protein complexes that recognized a diverse range of inflammation-inducing stimuli including both exogenous and endogenous signal e.g. microbial, stress and damage signals, and responded by activating caspase-1 and producing inflammatory cytokines IL1β and IL18. Recent reports supported Nod Like Receptor P3 (NLRP3) inflammasome activation in acne. Inflammasome protein NLRP3 and caspase1 were expressed in CD68+ macrophages and sebaceous glands in acne lessions in vitro and P Acnes triggered NLRP3 inflammasome activation in vitro resulting in IL1β released from sebocytes and monocytes cells but not from keratinocytes. Furthermore mature IL1β was expressed at the site of cutaneous inflammation, in the presence of macrophages surrounding the pilosebaceous units and in the sebaceous glands in acne lessions. P Acnes induced inflammasome activation depended on the internalization of the bacteria by antigen presenting cells, lysosomal maturation, activation of cathepsin B, generation of reactive oxygen species (ROS) and potasium efflux [10,21,22].

Toll Like Receptors



TLRs were the PPRs that recognized PAMPs presented on a variety bacteria and fungi. TLRs was a component of an innate immune response. TLRs were expressed at the interface with the environment where the host should defend against microbial threats. The activation of TLRs induced expression of costimulation mollecules and released cytokines that instructed the innate and adaptive immune response. TLRs directly activated host defence mechanisms that then combat the foreign invader [1].

The intracellular domain of TLRs had homology to the IL1 receptor and shared common signaling molecules of the Rel/NFkB pathway. TLRs expression by adipocytes, intestinal epithelial cells and dermal endothelial cells. IL4 acted to downregulate TLRs expression, Th2 cell in adaptive immune response inhibited TLRs activation [1].

There was the ability of innate immune system to use Toll Like Receptors/TLRs to recognized the microbial pattern and initiated immune response in cutaneous disorder. TLRs were the vital players in infectious and inflammatory diseases, and they were potential in the therapeutic targets. The ability of TLRs to combat disease already had beed utilized through the development drugs that acted as TLR agonist. A better understanding of TLRs would allow for the development of new therapeutic options for cutaneous inflammatory diseases such as acne [14].

Pathogens or danger signals were recognized by the innate immune system via surface pattern recognition receptors (PPRs) of which the most prominent group was the TLRs. The importance of the TLR mediated immune response in acne was supported by the presence of TLR2 expressing cells in innate immune cells in acne lessions. TLR2 was expressed in macrophages around pilosebaceous follicles and its expression was increased during the evolution of the disease. The expression of TLR2 and TLR4 was also increased in epidermal keratinocytes in biopsies of inflammatory of acne lessions. P Acnes had been shown to trigger both TLR2 and TLR4 in monocytes, keratinocytes and sebocytes in vitro. In addition, the bacterial components of P Acnes, peptidoglycans and lipoteicoic acid were considered to act as possible activators of TLR2 and TLR4 in acne [10,16].

P Acnes triggered an innate immune reaction via the activation of TLR2 and TLR4. TLR2 were a component of the innate cells immune system involved in host defense against invading microorganism. And their activation ultimatelly triggered the expression of immune response gene, including those coding for various cytokines and chemokines that stimulated recruitment of host immune cells. TLRs activated innate immune respons through keratinocyte, neutrophyl, macrophages, natural killer cells and dendritic cells. There were nearly a dozen different TLRs, but TLR2 and TLR4 appeared spesific for acne pathogenesis. Macrophages surounding the pilosebaceous unit with TLR2 receptor were histologically described in biopsiy material of patient with acne [18].

Olga was reported that stimulation TLR2 receptor of innate immunity cells by P acnes increased concentration of IL8 and IL12. P. Acnes activated several pathways that ultimately converged to activate Nuclear Factor (NF)ƙB transcription factor. Downstream released of inflammatory cytokines such as IL1α, IL6, IL8, IL10, IL12 and TNFα, that mediated pathogen destruction via effector cells [18].

Joane reported that the expression of functional TLRs in both interfollicular and infundibular human keratinocytes was consistent with the innate role for this cell in the sensing of and response to bacterial pathogen. The majority cutaneous pathogen were gram positive and therefore likely to initiate response via TLR2, as P acne exposure led to increase TLR2 as well as TLR4 expression in Normal Human Epidermal Keratinocytes/NHEK. There was TLRs expression predominantly in basal keratinocytes in acne lession. Viable stationary phase P acnes directly activated NHEK via TLR2 and TLR4 stimulating IL1α released, also IL8 released. So it was IL1α released from infundibular keratinocytes in response to P Acnes mediated TLR activation was an important step in natural complex hystory in acne lession. IL1α stimulated hypercornification leading to comedogenesis [31].

P acnes was microbial agents triggered inflammatory cytokines response via TLRs through NFkB activation. P acnes contributed to inflammatory nature of P acnes by inducing TLRs of monocytes to secrete pro inflammatory cytokine, including TNFα, IL1β, IL8, IL12. Interleukine/IL8 along with other P acnes induced chemotactic factors might play an important role in attracting neutrophyls to the pilosebaceous unit. In addition, P acnes released lipases, proteases and hyaluronidases which contributed to tissue injury [15].

The mechanism by which P acnes activated monocytes cytokine released was involved surface Pattern Recognition Receptors-TLRs2 and TLRs4 of monocytes as innate immune system,than contributed in inflammation at the site of disease activity. TLRs could discriminate between gram positive and gram negative bacteria. In acne lession TLR2 was also expressed on the cell surface of macrophages surrounding pilosebaceous follicles [33].

P acnes was one of the factor that contributed in the acne pathogenesis, that significantly increased in the pilosebaceous unit of the acne patients. P acnes was a variably and weakly gram positive bacteria. It was described as diphteroid or coryneform because it was rod shapes and slightly curved. A number unique features of P acnes cell wall and outer envelope further distinguished it from other gram positive bacteria. P acnes synthetized phosphatidyl Inositol, that unlike almost all other bacteria, but it was made virtually eukariotes. The peptidoglycan of P acnes was distict from other gram positive bacteria, caused a cross linkage region of peptide chain with L,L-diaminopimelic acid and D-alanine in which two glycine residues combined with amino acid and carboxy group of two L,L diaminopimelic acid residues. This lipopolisacharides acted as PAMPs that would initiate innate imunity through PPRs TLR2 receptor.

Jenny Kim research proved the spesificity response of monocytes in TLRs2, but not in TLRs4, TLRs6, TLRs1 that mediated by P acnes. This TLR2 activation induced IL12 production, a pivotal cytokines in activating Th1 cell immune responses in adaptive immunity and was of the one of the major pro inflammatory cytokines produced by monocytes in response to gram positive organisms. Than Th1 produced IL12 more caused IL12 overproduction that had been implicated in the development of tissue injury in certain autoimmune and inflammatory diseases [15].

In this research P acnes also induced the released of IL8, a cytokines that involved in neutrophyl chemotaxis in innate immunity. Released lysosomal enzymes by neutrophyls led to rupture of follicular epithelium and further inflammation.

The primmary event in inflammatory acne involved the disruption of the follicular epithelium and colonization of the follicles with P acnes with subsequent inflammatory reaction in the surrounding of epidermis. The detection of TLR2 in perifollicular region provided indirect evidence that TLR2 activation contributed in the pathogenesis of acne, suggesting that these cells promoted inflammatory response at the disease activity. This disease mechanism was supported by the colonization of TLR2 and its presence on monocytes and macrophages.

P acnes had been implicated as an important mediator of inflammation in the pathogenesis of acne. Pro inflammatory cytokines triggered by P acnes that mediated by TLR2 were unlikely had protective effect in acne, but it had harmfull effect in acne by promoting inflammation and tissue destruction [15].

Grech, et al research proved that P acnes also might stimulate expression of TLR4 in keratinocytes of the patients with acne vulgaris. There was a SNP/Single Nucleotide Polymorphisme of the TLR4 gene in acne vulgaris that was protective against the development of acne conglobata. There was no difference in ammount of the pro inflammatory cytokines released by peripheral blood mononuclear cells from acne conglobata wild type allele and SNP alelle. SNP alleles were found in healthy subjets and in acne patients [33].

Juggeau, et al research proved that P acnes induced TLR expression and that this mechanism could play essential role in acne linked inflammation. There was in vivo (from acne skin biopsi) increased TLR2 and TLR4 expression in the epidermis of acne lession and vitro (from cultured keratinocytes monolayers) increased of TLR2 and TLR4 expression in human epidermal keratinocytes as well as the increased of the expression and secretion of MMP9/Matrix metalloproteinase 9 by keratinocytes, which played a role in acne inflammation [34].

P acnes was involved in the induction of comedogenesis and maintenance of the inflammatory phase of acne. TLR2 expressed on the mononuclear inflammatory cell and possibly on keratinocytes and sebocytes was thought to be vital importance in mediating P acnes induced inflammatory response in acne vulgaris. Fathy's researched proved the TLR2 expression on the peripheral blood monocytes/PBM in inflammatory & non inflammatory acne patients and in control healthy subjects were signifficantly different. And proved TLR2 expression was significantly lower in acne inflammatory lession that was treated by isotretinoin. That data suggested that TLR2 expression on PBM was an important event in acne pathogenesis and targeting this mollecule (with isotretinoin) might be useful therapeutic goal in the future [6].

Nasser Duna reported that human keratinocytes through TLR2 and TLR4 produced IL18, like monocytes and macrophages did, being two major sources for this molecule. IL18 was pro inflammatory cytokines which than stimulated Natural killer cells and enhanced innate immunity as well as spesific Th1 immune response (adaptive immune response). IL18 acted directly to Natural killer cells to stimulate IFNɣ synthesis and upregulated their killing capacity. It was believed that than IL18 derived from keratinocytes might be also involved in cutaneous Th1 type immune response/adaptive immune response [25]. IL18 initially described as IFNɣ inducing factor, was a recently characterized cytokine that shared structural features with IL1 family of proteins. IL 18 induced IL8 gene expression and synthesis [25].

Protease Activated Receptor 2-Par 2 Genes

Toll Like receptor and Protease Activated Receptor 2 represented as two distict receptors related to innate immunity. PAR2 was expressed by almost cell types in the skin including keratinocytes, fibroblasts, sebocytes, endothelial cells, sensory neurons and inflammatory cells and was activated by endogenous and exogenous ligands.

In the human skin PAR2 was thought to regulate the homeostasis of the permeability barrier, keratinocyte cornification/differentiation, inflammation, pruritus, pigmentation and wound healing. In the

inflammatory acne lessions PAR2 expression was increased in sebaceous glands and in the suprabasal layers of the follicular epithelium linning the comedone. PAR2 activation had been shown to mediate sebocyte differentiation to induce lipogenesis and stimulated the pro inflammatory cytokines, HBD2 and LL37 in sebocytes and keratinocytes [10,21,35,36].

P Acnes produced proteases and via PAR2 triggering P Acnes had been shown to stimulate the expression of IL1α, IL8, TNFα, HBD2, LL37 and several matrix metalloproteibases (MMPs), including MMP-1,-2,-3, -9, -13 in the keratinocytes and sebocytes in vitro. In cultured human dermal fibroblasts P Acnes increased the expression of pro MMP 2 through TNFα [21,35].

MMPs were a family of zinc dependent endopeptidases, which degraded extracellular matrix components under both normal and inflammatory conditions and several MMPs including MMP-1,-2,-3 and-9 were up regulated in acne lessions. Facial sebum of acne patients also contained MMP1, MMP13 and tissue inhibitors of metalloproteinases TIMP1 and TIMP2 which were supposed to originate from keratinocytes and sebocytes. The upregulated MMPs might contribute to acne pathogenesis by inducing inflammation and tissue destruction. Increased MMP activity produced greater amount of degraded collagen in the lessional acne skin. Matrix breakdown was followed by imperfect repair, which was thought to result in acne scarring [35,37].

The roles of other endogenous proteases in acne had not been extensively studied. Granzyme B was strongly up regulated in inflammatory acne lessions. Tcs and Natural Killer had granzyme B in their granules that could destroy target cells. Tregs could also secrete granzyme A and B. Protease inhibitor 3 or skin derived antileukoprotease (SKALP), which was an elastase spesific protease inhibitor, was also upregulated in acne patient's skin as well as in the epidermis of psoriatic skin and in Netherton syndrome [38,39].

The serine proteases pro kalikrein 5 and kalikrein 7 were expressed in the interfollicular epidermis, the pillary canal and secondary sebaceous ducts. Their expression in acne was largely unknown in contrast to rosacea, in which the activity of kalikrein 5 was increased. Both kalikrein 5 and 7 control the activation of hCAP18, the expression of which was elevated in acne lession [40,41].

Other innate immune response in acne vulgaris was roled by PAR2 gene of the keratinocytes and sebocytes. PAR2 gene was the member of the unique G-protein-coupled receptor sub family with seven transmembrane domains and was expressed abundantly in various organs, including the skin. PAR 2 gene was associated with inflammatory diseases and cancers. Recent findings suggest that PAR2 was involved in various aspects of skin patophysiology, including melanin transfer, itch generation due to mast cell triptase, and epidermal barier homeostasis via the supressed secretion of lipid lamelae of keratinocytes. PAR2 binding to G-proteins had been shown in the epidermis, especially in the stratum granulosum and in the skin affected by the inflammation process. Pro inflammatory role of PAR2 in keratinocytes is a novel target for the treatment of cutaneous inflammation [42,43]. PAR2 modulated inflammatory responses, obesity, metabolism and act as a sensor for proteolytic enzymes generated during infections.

In innate immune response to various external stimuli and / or to endogenous pro inflammatory cytokines, epidermal keratinocytes of the skin release chemokines such as IL8, which recruited neutrophyls and lymphocytes into the skin and exacerbated lessional inflammation. During inflammation of the skin, Protease Activated Receptor/PAR2 in keratinocytes was activated by serine proteases such as leucocytes elastase and mast cell tryptase, released from infiltrating immune cells. PAR2 activation amplified inflammation via the upregulation of intercelluler cell adhesion molecule 1 (ICAM1) expression and released of IL8 from keratinocytes. Thus the activation of PAR2 and/or signaling pathways in the epidermis played pivotal role in inflammatory processes and their amplification in the skin [42].

During the course of cutaneous inflammation, keratinocytes released abundant amounts of IL8 in response to pro inflammatory cytokines such as IL1 and TNFα, which were amplified by the activation of PAR2, possibly triggered by upregulated kalikrein related peptidases from lessional keratinocytes and or by serine proteases from infiltrating inflammatory cells. PAR2 in the epidermis played pivotal role as a sensor, the activation of which led to the exacerbation of skin inflammation.

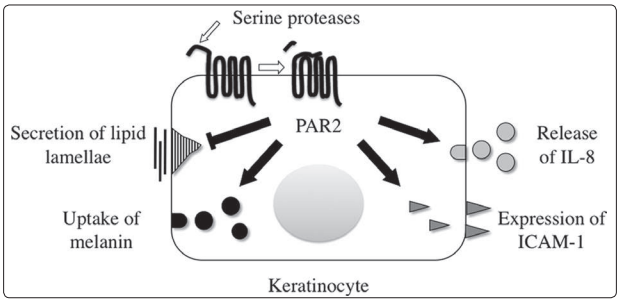


Figure: Activation and function of PAR2 in keratinocytes

Serine Protease cleaved the N terminal arm of PAR2 and the exposed new N terminal peptide could activate the receptor, The activation of PAR2 accelerated the release of IL8 from the cells and increased the expression of ICAM 1 and the uptake of melanin from melanocytes but supressed the secretion of lipid lamellae, which was essential for skin barier hemostasis [42]. PAR2 was activated by serine proteases that cleaved its N terminus and by synthetic peptides coresponding to the new N terminus [43].

Protease activated Receptor 2/PAR2 function as innate biosensor for proteases and regulated numerous functions of the skin. It was identified PAR2 expression inSZ95 sebocytes- an immortalized human sebaceous cell line, at both mRNA (gene) and protein levels. PAR2 signals primarily by coupling to the heterotrimetric G proteins, leading transient increase in intracellular Ca2+. Intracellular mobilization of Ca2+ in sebocytes suggesting that PAR2 was functionally active in human sebocytes. And intracellular Ca2+ mobilization indicated that P Acnes was the potent activator of PAR2 in sebocytes [36].

The small interfering RNA (siRNA)-mediated PAR2 AP treatment enhanced lipogenesis and sterol response Element Binding Protein 1/SREBP1 expression. SREBP1 was the master regulator lipid biosynthesis in sebocytes, suggesting the role of PAR2 in differentiation and lipogenesis in sebocytes. SREBP was the key of transcriptional factors that regulated genes encoding enzymes

of cholesterol and fatty acid synthesis in sebaceous glands, and SREBP pathway was known to be involved in the androgen and IGF1 mediated sebum production. PAR2 signal had important role in sebocyte differentiation and associated lipid synthesis, the major step of sebaceous gland differentiation process, that was supported by increased lipogenesis in the differentiated SZ95 sebocytes after stimulation with PAR2AP. Extracellular proteases signaled to sebocytes via PAR2 activation that contributed to sebocytes differentiation and lipogenesis [36].

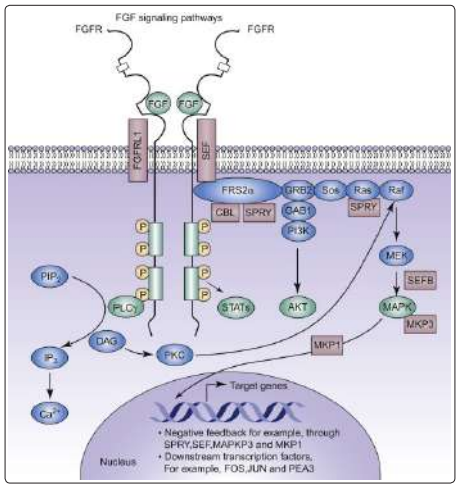
Moreover PAR2 gene induced cytokines (IL8 and TNFα) and anti microbial peptides Humanβ defensin 1/HBD2 transcription in sebocytes, indicated the role of PAR2 in the regulation of inflammatory and innate immune responses in sebaceous glands.

PAR2 expression was increased in sebaceous glands of acne lessions. PAR2 was expressed in SZ95 sebocytes and mediated differentiation, lipogenesis (lipid synthesis), inflammation and innate immunity in response to P Acnes which had protease activity. PAR2 expression was increased in sebaceous glands of acne lessions. Activated PAR2 on sebocytes and induced lipogenesis partly via PAR2 signals. PAR2 might be as therapeutic target for sebaceous gland disorders such as in acne [36].

PAR2 played important role in the pathogenesis of acne by inducing inflammatory mediators in response to proteases secreted from P acnes. P. Acnes had been known to produce various exogenous protease. Protease elicited cellular response. At least in part, via Protease Activated Receptor 2/PAR2, which was known to mediate inflammation and immune response. Protease from P Acnes could activate PAR2 gene on keratinocytes and induced pro inflammatory cytokines, anti microbials peptides (AMPs) and matrix metalloproteinnase (MMPs) via PAR2 signalling. The protease activity and PAR2 expression were increased in acne lessions P. Acnes induced calcium signaling in the keratinocytes via PAR2 and stimulated the m RNA expression of interleukine IL1α, IL8, TNF, HBD2, LL37, MMP 1-2-3-9-13 in keratinocytes [35].

So PAR2 gene on sebocytes and keratinocytes caused defect differentiation, enhanced lipogenesis and SREBP1 expressions, induced cytokines and human beta defensin 2, induced matix metalloproteinnase in keratinocytes in response to P Acnes [35].

Fibroblast Growth Factor Receptor 2-Fgfr2



FGFR2b signalling in dermal-epithelial interaction was for skin appendage formation, pilosebaceous follicle homeostasis, comedogenesis, sebaceous gland proliferation and lipogenesis. Androgen mediated upregulation of FGFR2b signalling in prone skin appeared to be involved in the pathogenesis of acne vulgaris. In keratinocytes, IL1α stimulated fibroblasts to secrete FGF7 which stimulated FGFR2b that mediated keratinocyte proliferation. FGFR2b was importance in sebaceous gland physiology, down regulated or attenuated FGFR2b signalling by isotretinoin caused sebaceous gland atrophy. Insulin like growth factor-1 (IGF-1), the mediator of growth hormone during puberty, interacted with androgen dependent FGFR2b signalling and linked androgen and FGF mediated signal transduction important in sebaceous gland homeostasis [46].

Elevated level the pro inflammatory cytokine IL1α was an important inducer of keratinocytes proliferation, hyperkeratinization and reduced desquamation of comedo by stimulated fibroblast to secrete FGF7 which stimulated FGFR2b to mediate keratinocytes proliferation [46].

IL1α stimulated fibroblast to secreted Fibroblast Growth Factor7/ FGF7 and FGF10 , that then stimulated FGFR2b that mediated keratinocytes differentiation. FGFR2 signalling in dermal epithelial interaction was important for skin appendages formation, pilosebaceous follicle hemostasis, comedogenesis, sebaceous gland proliferation and lipogenesis. Increase FGFR2 signalling played a major patogenic role in follicular hyperkeratinization and sebaceous gland hypertrophy in acne [46].

Some anti acne attenuated FGFR2 signalling. FGFR2 was a receptor for fibroblast growth factor. FGFR2/Fibroblast Growth Factor Receptor 2 also known as CD 332 was a protein that in humans was encoded by the FGFR2 gene residing 1q on chromosome 10. FGFR2 signalling proposed to be involved in acne pathogenesis an explain acne lession in Apert syndrome and unilateral acneiform nevus associated with gain of function point mutation of FGFR2 [46].

There was interaction between androgen - Insulin Growth Factor1 and FGFR2 signalling for sebaceous gland homeostatis and development. IGF1 stimulated 5α reductase, that then stimulated sebocytes proliferation more than differentiation and stimulated lipogenesis. FGFR2 stimulated sebocytes differentiation more than proliferation. Androgen was important in increased the size of sebocytes and increased sebum production [46].

IL1α in comedo increased FGFR2 signaling and induced keratinocytes proliferation - hyperkeratinization and reduced desquamation of comedo formation. And FGFR2b upregulated IL1α that was involved in hyperproliferation of infundibular keratinocytes and comedo formation [46].

Androgen increased the expression of FGF7 and FGF10, than mainly FGF7 bound to its epithelial FGFR2b receptor on sebocytes and keratinocytes of follicle duct. FGFR2 activation led downstream/ feed back of IL1α that induced terminal differentiation of sebocytes, upregulated MCSR and MCR dependent lipogenesis.

FGF7 bound to FGFR2b, induced TGFα and EGFR in keratinocytes. Androgen upregulated EGFR that augmented FGFR2b effect by increased G coupled mediated EGFR transactivation [46]. So

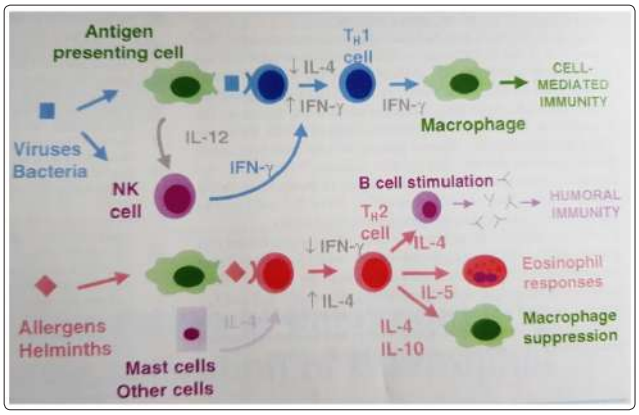
androgen increase FGF7/10, FGFR2b and IL1α pathway to induce hyperproliferation and activation of infundibular keratinocytes and sebocytes [46].

Androgen dependent FGFR2 mediated, could release IL1α by infrainfundibular keratinocytes. IL1α was the primary stimulus leading to comedogenesis, secretory keratinocytes activation inducing the perifollicular infiltration by T cell and macrophages and IL1α initiated immune response. Activated IL1α receptor Associate Kinase/TRAKs that were involved in IL1rec/TLR mediated signal transduction process would regulate innate and adaptive immune response. Sebocyte was precursor stem cell like keratinocyte. Sebocytes produced IL1α [46].

Adaptive Immunity In Acne Vulgaris Inflammation Process

Adaptive immune response referred to antigen spesific immune response But in acne vulgaris adaptive immunity was caused by non spesific antigen that was involved in early stage of inflammation (less than 6 hours), but in the later stages (more than 6-72 hours) adaptive imunity to spesific antigen then developed too following innate imunity to spesific antigen reaction.

The adaptive immune response was more complex than the innate. The antigen first should be processed and recognized. Once the antigen had been recognized, the adaptive immune system created an army of immune cells specifically designed to attack that antigen. Antigen spesific T and B lymphocytes mediated adaptive immune responses. An adaptive immune response corresponded to the type and strength of the innate response. Activated B cells secreted immunoglobulins and were involved in humoral immunity, whereas T cells were involved in cell mediated immune response [45,46].



Firstly, there was an ability of innate immune system to use TLR2 receptor to recognized microbial pattern and initiated immune response in cutaneous disease [9]. TLR 2 receptor induced inflammatory response and the development of antigen spesific adaptive immunity [6]. T1 lymphocytes via CD4+ and B cells antibody secretion binding P acnes were responsible in adaptive immunological reaction in acne vulgaris [3,4].

In the moderate and severe inflammation, there was disruption of the duct and macrophage giant cell foreign body reaction [3]. An amplification phase via antigen dependent T cell responses to other comedonal components for example *P acnes*, might then developed [4].

P acnes dependent antibody (ADCC) was adaptive immune response, through B cells that produced antibody to P.acnes. Circulating antibody to *P acnes* were elevated in patient with severe acne [1].

In the late phase of inflammation, *P acnes* dependent T cell lymphocyte response could been found, there were variations in Cell Mediated Immune Response depended on individual microflora sensitization [1,4]. It was an other adaptive immun response in acne vulgaris inflammation through T cell lymphocytes.

It had been found that delayed resolution of inflammatory acne lession took place due to opportunities of P acnes to stimulate the production of antibodies and histamine vasoactive peptides, as well as their resistance to neutrophilic and monocytic phagocytosis [18].

IL18 was proinflammatory cytokine that was produced by binding bacteria through TLR2 and TLR4 keratinocytes, monocytes and macrophages would stimulate NK cell in innate immunity and cutaneous Th1 cell in adaptive immunity [25]. The new finding, especially that was the IL17/Th17 pathway that cotributed significantly to inflammatory response in acne adaptive immunity response that involved in early stages of acne lessions [18].

Hana research was reported that it was inflammation skin reaction of the skin of acne vulgaris patients, particularly regarding activation of adaptive immune response in particular T cell subsets. There was activation of the IL17/Th17 axis, which may together with IL1 be an essensial component in the immunopathogenesis of acne. The IL17/Th17 axis was activated in clinically early stage acne lessions. The inflammatory infiltrates contained the CD4+ that were Th1, Th17 and Tregs, and also CD68+ macrophages, mature CD83+ DCs cells and CD8+ T cells [10].

The most prominent function of IL17 was neutrophyl chemotaxis mediated by the production of granulocyte colony stimulating factor (G-CSF) and chemokines such as IL8/Cxcl8. IL17 also induced chemo attractans eg. Ccl20, Gm CSF for lymphocutes, DCs and monocytes and modulate the production of AMPs. Importantly IL17 synergized with other inflammatory cytokines in particular TNFα, IL1β, IL22 and IFNγ [10].

In addition of its potential function to attract the neutrophyls in early phases of acne inflammation, IL17 accounted for the increased expression of AMPs/anti Microbial Peptides in acne lession. IL17 together with IL1 and TNFα induced the production of several MMPs/Matrix Metalloproteinnases, whose expression was also increased in acne [10].

Acne pustules contained large number of neutrophyls which could for their part secreted more IL17 and intensified inflammation. In more severe severe form of acne with nodular and cystic lessions there could be very intensive IL17 reaction because of massive neutrophilic inflammation [10].

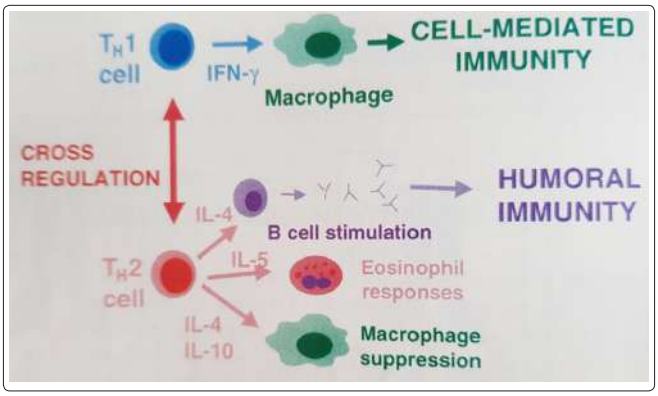
As well as by CD4+ cells and neutrophyls, IL17 could also be produced by several other types including Tc cells, group 3 innate lymphoid (ILC3) cells, gama teta cells, NK cells, Mast cells and macrophages. Keratinocytes were principal cellular target of IL17A, but IL17 receptors were constitutively expressed on fibroblasts, osteoblasts, chondrocytes, macrophages, DCs and endothelial cells. It was noticed that IL17 expressing cells were mainly lymphocytes

with minority of neutrophyls and mast cells [10].

The expression levels of TGFβ and FOXP3 m RNA were lower in acne patients uninvolved skin than healthy normal skin [10].

P.acnes induced IL17 expression in human peripheral blood mononuclear cells (PBMC), that induced naive CD4+ T lymphocytes to differentite/expressing into Th 17 cells and Th1 cells expressing IFNɣ, IL1α, IL6 and TGFβ/transforming growth factor regulated P. Acnes and induced IL17 response as they did in other system. Finding IL17 and Th17 cells proved the possibility that acne was not uniquely a Th1 mediated disease, suggest that immune acne immune responses were more complex than thought previously. In addition showing that P. Acnes induced the production of IFNɣ/IL18 , a key cytokine marker for Th1 cells, it was demonstrated that P.acnes modulated IL17 and IL22 production as well as the expression of Th1 receptors on P+MBCs [29].

T Cells [10,11,46]



T cells were divided into two main groups, CD4+ helper T cells (Th) and CD8+ cytotoxic T cells (Tc) and they identified antigen-derived peptides by their antigen receptor. Th cells recognized antigens bound to MHC class II molecules expressed on APCs. Most Tc cells recognized the endogenous antigens presented by class I MHC mollecules, which were expressed normally on all nucleated cells. Recognition of foreign antigen-derived peptides by naive T cells led to a process that included massive proliferation and differentiation into distinc T cell subsets according to the corresponding cytokine profile. Th cells helped B cells to produce antibodies, activated Tc cells, and recruited and activated other immune cells. Tc cells acted as killer cells, but like CD4+ cells, they could also exert regulatory functions and produced different cytokines [10].

Chemokine mediated migration of T cells

Antigen inexperienced T cells were termed naive and could be identified by the expression of three cell suface proteins. CD45RA (an isoform of the pan leukocyte marker), L-selectin and the chemokine receptor CCR7. These T cells migrated efficiently to secondary lymphoid organ (e.g. Lymph nodes), where they might make contact with antigen bearing DC from periphery. Once activated by DC presenting antigen, T cells then expressed CD45RO, were termed memory T cells, and appear to express a variety of adhesion molecules and chemokines receptors that facilitated their extravasation from blood vessels to inflammed peripheral tissue. A spesific subset of CCR7 negative and L-selectin negative memory T cells had been proposed to represent an effector memory T cells subset that was ready for rapid development at peripheral sites termed of cytotoxic activity and ability to mobilize cytokines [1].

Although chemokines were both secreted and soluble, the net positive charge on most chemokines enabled them to bind to the negatively charged proteoglycans such as heparan suphate that were presented on the lumenal surface of endothelial cells. Thus, chemokines were presented to T cells as they rolled along the lumenal surface. After ligand binding, chemokine receptors sent intracellular signal that led to increases in the affinity and avidity of T cells integrins such as LFA-1 and very late antigen-4 (VLA-4) for their endothelial receptors ICAM-1 and vascular adhesion molecules-1 (VCAM-1). Only a few chemokine receptors (CXCR4, CCR7, CCR4 and CCR6) were expressed in the sufficient levels on resting peripheral blood T cells to mediate this transition. With activation and interleukine IL2 stimulation, increased number of chemokine receptors (e.g. CXCR3) were expressed on activated T cells, making them more likely to respond to other chemokines. In several different systems, inhibition of spesific chemokines produced by endothelial cells dramatically influenced T cell arrest in vivo and in vitro [1].

Th cells were the central players in adaptive immune responses. Many types of specialized Th cells had been identified. The main Th cell lineages were Th1, Th2, Th17 and regulatory T (Treg) cells. Th1 cells promoted cell mediated immunity by activating macrophages and CD8+ T cells to kill viruses and other intracellular pathogens. Th1 cells also contributed to the pathogenesis of autoimmune diseases. Th2 responses were critical for IgE production and activation of eosinophyls, mast cells and basophyl. Th2 were important for the elimination of helminthic parasites but now they were known better for their role in the pathogenesis of astma and other related allergic conditions. Th17 cells recruited and activated neutrophyls and contributed to their host defence against extracelluler bacteria and fungi. They contributed to chronic inflammation associated with many inflammatory and autoimmune disorders. Treg cells were immunosupresive and maintained self tolerance, prevented autoimmunity and controlled immune responses during infection and cancer. In addition to four major lineages, other potential new Th lineages had been proposed including Th3, Th9, Th22, follicular Th (Tfh) cells and type 1 regulatory (Tr1) cells. Since the signature cytokines produced by these cells were also the products of Th1/Th2/Th17/Treg cells and the trascription factors, they express were not unique, it was possible that these cells represent subsets of the four main lineages [11,46].

In addition of heterogenicity between different Th lineages, individual T cells within same lineage might display different pattern of cytokines production and express transcription factor differentially. Moreover, Th cells were capable to switch from one lineage to another or to mixed phenotype suggesting that Th cells were plastic. At early stage of The differentiation, each Th lineage could be plastic but at later stages only the majority of Th17 and Treg cells might alter their cytokine production profile [46].

The prevalence of T cells and macrophages in early stage acne lessions indicated their key role in inflammation of acne. CD3+ and CD4+ T cells were the major immune cells in the inflammatory infiltrates of early stage (6-72 hours) acne lessions and the number of CD8+ T cells increased with the age of the lession. Additionally the numbers of CD4+ T cells (most of which were CD45RO+ memory/effector cells) and CD68+ macrophages were elevated in the uninvolved follicles of acne patients, and in acne lessions less

than 6 hours old compared with healthy skin controls, indicating that adaptive immune reactions in acne begun even before microcomedo formation [12].

Spesific cell mediated immunity to P Acnes increased during the course of severe inflammatory acne acne. P Acnes had been shown to induce T cells proliferation. In addition, P Acnes reactive CD4+ T cells with an IFNɣ cytokine profile had been found in early stage papular inflammatory lessions suggesting the presence of Th1 cells in acne. Recently cells with Th1 and Th17 profile had been found in inflammatory acne lessions. Moreover, P Acnes had been shown to trigger Th17, Th1 and mixed Th17/Th1 responses in peripheral blood mononuclear cells (PMBCs) in vitro [23].

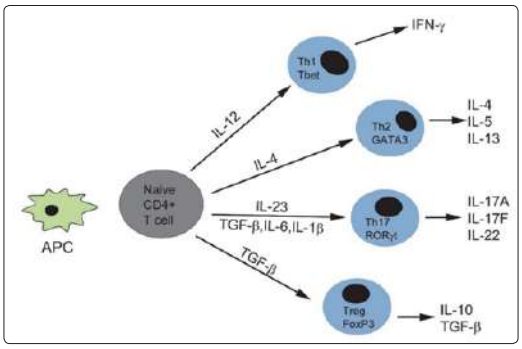


Figure: The differentiation of T helper cells. The interaction of antigen presenting cells (APC) with naive CD4+ T cells in the various cytokine milieus induced certain transkription factors in the T cells (Tbet, GATA3, RORyt, FoxP3), which directed differentiation into Th1, Th2, Th17 and Treg cells

Humoral Immunity

As well as an increase in number of Th cells, the number of B cells also seemed to correlate with increased severity of acne in the peripheral blood. The antibody on the B cells recognized the spesific antigen and eventually with the assistance of Thelper2 cells, the B cells differentiated into antibody producing plasma cells. Secreted immunoglobulin (Ig) bound to antigens and then neutralized them or facilitated phagocytosis or complement activation. Complement-fixing antibody titers to P Acnes were in the levels found for most adults with mild acne but titres increased with the severity of inflammation. Increased total IgG had been observed in patients with severe acne and antibody titers to other skin organisms such as Staphylococcus epidermidis had been noticed. In severe acne elevated antibody response was directed against carbohydrate structures in the P Acnes cell wall. Unfortunately the studies about humoral immunity in acne had not been publised recently and therefiore it was difficult to evaluate the role humoral immunity in pathogenesis acne [47-50].

In study was reported the elevated IgE levels that related with clinical severity in a group, but another group was found no changed in total IgE levels [3]. Female had better defence mechanism than male against P acnes [3]. Most acne patient had no misfit immunological reaction [3]. There were no circulating immune complexes in acne sera patients [3]. Skin testing with heat killed suspensies of *P acnes* demonstrated that subject with severe acne produced a greater inflammatory reaction at 48 h than other subject, suggested that host response might been important [3]. Changing in neutrophyl chemotaxis might been the secondary event [3]. *P acnes* polypeptide were detected in serum of the acne patients but were not in normal individu [3]. Acne fulminant showed exaggerated delayed hypersensitivity reaction to *P acnes* [3].

Stages In Acne Vulgaris Inflammation Process

Several converging lines of evidence indicated that inflammation might be present throughout the development of acne lessions, both during the later stages where inflammatory papules, pustules and nodules were present, and also during the early stages of lession development in microcomedos and comedos [18].

In the early non inflammed and inflammed lession had shown that there were activation of the classical and alternative pathways [3]. And there were the type 4 immunological reaction to a non spesific antigens in the prior of obvious duct ruptured [3].

The majority comedones clearly represented a dermal pool of pro inflammatory IL 1α [4]. Spongiosis of the pilosebaceous follicle wall was the feature of early inflammation changed, this could led to leakage of comedonal IL 1α into the epidermis [4]. The consequence was the activation of dermal microvascular endhothelial cells, selective accumulation of antigen non spesific to mononuclear cells and inititation of antigen independent cutaneous inflammation that consistent with the hystological features of early inflammation in acne [4].

In the later, in the moderate and severe inflammation , there was disruption of the duct and macrophage giant cell foreign body reaction [3]. An amplification phase via antigen dependent T cell responses to other comedonal components for example *P acnes*, might then developed [4]. The intensity and duration of the subsequent cell mediated response would been depended on many factors, including the degree of individual sensitization to their cutaneous microflora [4]. Following the disruption of cell wall, neutrophyl would been attracted into the duct by microbial chemotactic factor, that was proved by a study that demonstrated the capability of *P acnes* to attract neutrophyl in vitro [4]. So *P acnes* might caused inflammation because this organism had been shown to secrete chemotactic factors and the chemotactic activity had been shown in comedones [1]. Low mollecular weight chemotactic factor did not require serum complement for activation and because of its small size, it could probably escaped from follicle and attracted polymorphonuclear leukocytes [1]. If polymorphonuclear leukocytes enter the follicle, they could ingested *P acnes* organisms, resulted the release of hydrolytic anzymes which in turn, might been importance in producing follicular epithelial damage [1]. Polymorphonuclear leukocyte ingested *P acnes* was anti *P acnes* dependent antibody (ADCC) [1].

Bacterial cell walls fractions of *P acnes* were a potent chemoattractan for polymorphonuclear and mononuclear cells, could also produced prostaglandine like substance, that acted as non streroidal anti inflammatory drugs, that had small anti acne effect [3].

It was likely but was not been proven that *P acnes* played an important role in acne inflamation [4]. Whether *P acnes* played a role in the initiation of inflammation in acne was questionable since it had not been colonized at all of the early lessions. Nevertheless, there was an increasing in the number of lessions colonized by *P acnes* following early inflammatory change. *P acnes* were a potent adjuvant that induced a chronic inflammatory tissue response because it was resistant to phagocyte killing and degradation [4]. So *P acnes* caused

chronic inflammation process because of its resistance to phagocyte cell and could not been degraded [4].

In the late phase of inflammation, *P acnes* dependent T cell lymphocyte response could been found, there were variations in Cell Mediated Immune Response depended on individual microflora sensitization [1,4]. Circulating antibody to *P acnes* were elevated in patient with severe acne [1]. Patients with severe acne were significantly more sensitized to *P acnes* than normal individuals, and the overall immunological status of patients were elevated compared with acne free individuals of the same age [4]. But this observation did not provide direct evidence for a pathogenic role of *P acnes* in initiating inflammatory acne and might merely reflected an increased exposure of patient to the organism as result of their condition and might played a role in the exacerbation of chronic inflammatory response [4].

Inflammation That Provoked By Altering Sebum Composition

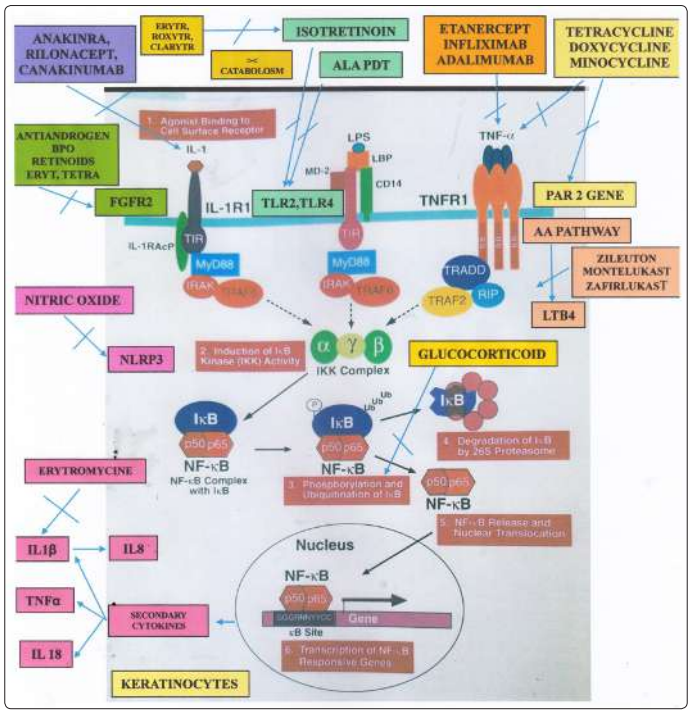
Lipid that got into dermis when the duct ruptured acted as an irritant and some lipids like linoleic acid could down regulated neutrophyl oxygen metabolism and phagocytosis, in acne patient there was decreased linoleic acid concentration resulted more inflammation [3]. Inflammation was resulted from the production of free fatty acid and it showed that *P acnes* was the main source of follicular lipase that hydrolized trigliserida to free fatty acid [1]. The sebolemmal sheath accumulated inspisated sebum and formed a firm calculus which eroded the duct wall and contributed to inflammation [3].

DHT was the main driver of androgen induced sebum production of the skin. DHEA was another hormone for increasing sebaceous gland activity. Increasing DHEA secreted in adrenarche (puberty) could increased sebum synthesis. Sebum rich environment made skin *P acnes* grew and caused inflammation around follicle due to activation of innate immune system that increased pro inflammatory mediators: cytokines IL1α, IL8, TNFα and Leukotrien LTB4 production, than cytokines attracted various immune cells to the hair follicles (neutrophyls, macrophages, Th1 cells). IL 1α stimulated and triggered keratinocytes activity and reproduction which turned fueled comedo development [51].

P acnes provoked inflammation by altering sebum composition [51]:

1. *P acnes* oxydated squalene that Activated NFkB pathways and consequently increased IL 1α levels. The Increasing of 5 lipooxygenase enzym that responsible in arachidonic acid pathway- leukotriene B4 (LTB4) than promoted skin inflammation by acting on peroxisome proliferator activated receptorα (PPARα). PPARα increased activity of activator protein (AP1) and NFkB led to recruitment of inflammatory cells. AP 1 inflammatory cascade led activation of matrix metalloproteinase which contributed to local tissue destruction and scar formation [27]. Leukotrien B4 was a family of eicosanoid inflammatory mediators produced in leukocytes by the oxidation of arachidonic acid (AA) and the essential fatty acideicosapentaeoic acid (EPA) by the enzym arachidonate 5 lipooxigenase. The inflammatory chemical leukotriene B4/ LTB4 was known to upregulate sebum production [51].
2. *P acnes* Hydrolized triglycerides to pro inflammatory free fatty acid through lypase enzyme of *P acnes*. FFA spur production of antimicrobial peptides / AMPs (Such as Human β defensin 1/ HBD1, cathelicidine, human β defensin 2/HBD2) thus leading to further inflammation. That inflammation in acne lession was broken in the deep layer and formed nodules [51].

Anti Immunological Reaction Therapy in Dermatology Practice



Cytokines and other pro inflammatory mediators were emergency mollecules, designed to be released locally and transiently in tissue environments. When they were released persistently, the result typically was the chronic diseases such as acne vulgaris and other chronic inflammatory diseases. One potential way to treat such diaseases were with cytokine / chemokine / leukotriene antagonists, other drugs that targeted cytokines or other proinflammatory mediators, or drugs that acted in cytokines mediated pathways.

Glucocorticoid

Corticosteroid had antiinflammatory effect, this effect was discovered by Hench in 1950. Glucocorticoids inhibited as broad a range of proinflammatory effects as primary cytokines induced. Glucocorticoid had antiinflammatory activity and had some potential complication in pharmacologic uses. A link between glucocorticoid and primary cytokines were made through the discovery that glucocorticoid increased the gene expression of 1ƙBα, thus serving to replace 1ƙBα degraded by proteasomes after phosphorilation and ubiquitination and ultimately to sequester NFƙB dimers in the cytoplasm. Effectively, then glucocorticoids tip the balance between sequestered and active NFƙB by inducing 1ƙBα synthesis, while primary cytokines promoted inflammation by liberating NFƙB from its association with 1ƙB. Thus glucocorticods attacked the central NFkB pathways by which primary cytokines work, had antiinflammatory effect, then decreased producing IL1β, TNFα and IL18, that caused persistent inflammation and chronicity of acne vulgaris [1].

Glucocorticoid could lead to the inhibition of MHC class II antigen presentation pathways, which could downregulate immune responses leading to tissue injury and could also contribute to immunosupression [1].

Glucocortikoid was upregulated TLR [10]. The activation TLR on macrophages by microbials ligands upregulated iNOS, which also result rapid generation of NO (caused decreasing of IL1β) and powerfull microbial activity. Macrophages used this mechanism to contain some infectious organisms that were not susceptible to PMN attacted such as mycobacteria, fungi, parasit and P acnes that had been resistant to phagocytosis. The stimulation of monocytes by activated T cells also led to generation of NO [1].

Topical steroid acted as antiinflammatory, immunosupresive, anti mitogenic were due to their ability to exert multiple effects on the various function of the leucocytes, epidermal and dermal cells. Topical steroid decreased erythema (produced transient vasoconstriction) and irritacy that could be caused by topical tretinoin. Topical steroid avoided the possibility of hypertrophic scar and keloid that still could be caused by daily application of 0,5% topical tretinoic [3].

Steroid mainly acted in central NFKB pathway to stop the production of primary cytokine positive loop activation that caused persistent inflammation (mainly caused by IL1β & TNFα) and secondary cytokine (IL18) production. Steroid mainly acted in central NFKB pathway by increasing the gene expression of 1kBα thus serving to replace 1kBα degraded by 26Sproteosome after phosphorilation and ubiquitination and ultimately to sequester NFkB dimers in the cytoplasm as inactive complexes (NFkB-1kBβ tight bounding). Steroid tip the balance between sequestered and actived NFkB by inducing 1kbα synthesis. Primary cytokine (IL1β,TNFα) then promoted persistent inflammation by liberating NFkB from its association with 1kβ→increasing NFKβ released and nuclear translocation to nucleus for transcription→further increased secondary cytokine production (IL18), caused increasing of inflammation [1]. In non stimulated cells NFkB heterodimers formed from p65 and p50 subunits were in active because they sequester in the cytoplasm as a result of tight binding to inhibitor proteins in the 1kB family. So NFkB did not release and there were no nuclear translocation for transcription, caused decreased cytokine production (IL1α,IL1β, TNFα) so decreased comedo formation and inflammation [1].

Steroid decreased tissue destruction and scar formation by blocking the production of all arachidonic acid derivates (eicosanoids) through induction the production of lipocortin. Steroid blocked arachidonic acid pathway that caused decreased production of leukotriene B4 (LTB4) which caused decereased activation of peroxisome proliferator activated receptorα (PPARα) and decreased activity of activator protein (AP1) inflammatory cascade, that finally caused decreased production of matrix metalloproteinase, so caused decreasing of local soft tissue destruction and scar formation [3].

The currently accepted general model of steroid action incorporates three major steps receptor binding, synthesis of spesific m RNA (transcription) and the synthesis of protein. Hydrophobic glucocorticoid mollecules diffuse across the plasma membrane of the cell and reversible bound to spesific receptor protein that was presented in the cytoplasm. This hormones (glucocorticoids) binding to the receptor caused increased DNA binding affinity due to allosteric changed in the structure of the receptor, leading to the accumulation of the steroid receptor complex in the cell nucleus. Gene transcription could be modulated by the hormonal complex binding to certain sequences on nuclear DNA termed hormone response element (HREs), which resulted the production of the new proteins by RNA mollecules using the new DNA complex as a template. Some of the new protein produced included lipocortin, Interleukine 1 and lymphokines (e.g. Interleukine2). Lipocortin a family of glycoproteins, plays an important role in regulating the activity of phospholipase A2, which subsequently effected the production and released of arachidonic acid, which was the precursor of leukotrienes and phospholipids [1,3].

Isotretinoin/Retinoid (TLR2 Antagonist)

Retinoid/Isotretinoin (13 cis retinoic acid) was related to vitamin A. It did not directly affect either innate and adaptive immune responses in newly acne response, except for macrophages, which were abundant in acne patients skin. This implies that once single acne lession was formed, isotretinoin did not effectively stop the inflammation, because so many other cell immunological responses could not be inhibited. Retinoid down regulated TLR2 expression of the macrophage but not TLR4 resulted decreased of IL1β (so it acted as TLR2 antagonist) in patient non lessional skin/uninvolved skin. Isotretinoin restored the low levels of S100A7 in uninvolved skin of acne patients to the levels of normal skin control. Isotretinoin and lymecyline both diminished the relative abundance of P Acnes in acne areas and the decreased dominance of P Acnes increased the diversity of microbiota [10,20].

Retinoid regulated differentiation, reduced hyperproliferation and inhibited leucocyte migration [10].

In contast with Hana 2016 expectation that isotretinoin had no effect on adaptive immune responses in newly form acne lession, Ellias et al 2008, Mucida et al 2007 and Xiao et al 2008 proved that isotretinoin inhibit inflammatory Th17 responses in early acne lession, promoted regulatory T cell responses, isotretinoin influenced Th17/Treg balance in acne patient skin. In hana tesis proved that that were non significant statistically reduction of IL17A and IFNγ but not IL 10 level which resulted decreasing of inflammation [10].

In Beata,et al research proved that isotretinoin was efficient to treat acne vulgaris. Isotretinoin caused a decreased in pro inflammatory cytokine level of IL1α, IL1β and TNFα patients sera concentration. The best therapy scheme was the use of constant drug dose 0,4-1,0 mg/kg bw/day without modification during treatment. Decreased measurement of IL1α, IL1β and TNFα sera concentrations could be assesed in parallel to the improvement of the clinical condition and constituted a good indication of the efficiency of isotretinoin treatment. In this research IL8 was not good evaluation parameters for isotretinoin treatment [53].

Isotretinoin (13-cis-retinoic acid) was regarded as prodrug, because retinoid itself did not activate retinoic acid receptors (RARs) or retinoid X receptor (RXRs). These receptor was activated by isotretinoid's active metabolites, including all trans retinoic acid (ATRA). Isotretinoin influenced cell cycle progression, cellular differentiation, cell survival and apoptosis, resulting in reduced sebum production and alteration of composition of skin surface lipids and attenuated growth and proliferation of infundibular keratinocytes. Isotretinoin had no direct anti microbial effect, but caused the reduction of surface and ductal P Acnes as a result of altered microenvironment due to reduced sebum production and smaller sebaceous glands [10,20].

The anti inflammatory effect of isotretinoin were also considered important. Isotretinoin and other retinoid down regulated TLR2 expression on monocytes. Furthermore, retinoids induced Treg differentiation and down regulated Th17 differentiation and neutrophyl migration, but is was unknown whether this mechanism were modulated by isotretinoin in acne. Isotretinoin also down regulated the expression of MMPs -9 and -13, which played a central role in inflammatory matrix remodelling. Finally retinoids stimulated the released of GM-CSF, monocyte chemotactic protein-1 (MCP-1) and IL10 in cultured monocytes and macrophages [10,54].

Retinoids attenuated the FGFR2 pathway at several regulatory levels of the signal transduction cascade critical for cycle cell control, cell proliferation, differentiation and lipogenesis. Isotretinoin induced TGFβ (from T cell) caused terminating of keratinocytes and sebocytes activation cycle in acne vulgaris. Retinoid acid (9cis, 13 cis, all trans retinoic acid) increased TGFβ in dermal papillary caused inhibition of P53, increased of growth inhibition and increased hair follicle regression. Isotretinoin decreased FGFR2 signaling caused primary deactivation signal of activated keratinocytes and sebocytes in differentiation. Retinoids attenuated the FGFR2 pathway at several regulatory levels of signal transduction cascade, that caused decreased of cell cycle control, cell proliferation and differentiation and lipogenesis. Downstream FGFR2 signalling primary deactivated signal of activated sebocytes and keratinocytes [46].

Set Orlow, dermatologist from New York university told that topical retinoids unclogged pores by decreasing stickness of keratinocytes inside the follicle and increasing cell turnover. Isotretinoin was highly effective at shutting down the sebum production, shrinked the sebaceous gland and might have effect on keratinocytes shedding. Side effect of retinoid was redness, sore skin with blister that sensitive to the sun. Oral isotretinoin side effects were hyperlipidemia, abnormal liver function test, lost of the night vission, deppression, suicidal thought, teratogenic. Friedman also reported that retinoid had anti inflammatory effects by supressing TLRs [4].

Topical tretinoin mechanism of actions were normalized differentiation and keratinization, prevented keratin plug formation. The action in the epidermis was restored of normal keratinization in conditions in which this was disurbed. It enhanced DNA synthesis in the germinal epithelium and increased the mitotic rate. It also had regulating effect on epidermal cell differentiation, leading to a thickening of granular layer and a normalization of keratinization in parakeratosis. Retinoic replaced of disorganized dermal collagen fibers, caused an increased in epidermal thickness and returned to a more uniform size and electron density of the basal and spinous keratinocytes. Retinoic decreased sebum production by reducing the size of sebaceous glands, in which the profoundly inhibited sebum excretion and affected comedo formation. Isotretinoin reduced 5α reductase activity in human liver and skin and reduced binding capacity of the androgen receptor in back skin [1,3].

Binding proteins for retinoids were widely distributed throughout the body in many cell types. Cellular retinoic acid binding protein II (CRABP II) predominated in the skin, and could be found in the keratinocytes and fibroblasts. Following transport to the nucleus, retinoid bound to retinoic acid receptor (RAR) and retinoic X receptor (RXR). Receptor interaction with DNA response elements stimulated promoter regions to induce transcriptional and translational activity. The resultant was protein synthesis, that in turn mediated structural and functional activity of the cells and modulated transcription of other genes [3].

Tretinoin was bound to nuclear receptor (RARs and RXRs) that acted as ligand activated transcription factor, Retinoid receptor complex modulated the transcription of spesific gene (DNA). Tretinoin receptor complex antagonized various transcription factors that was involved in proliferative and inflammatory responses such as AP1 (activator protein 1) or nuclear factor IL6 (NF-IL6) by competing for commonly required coactivator proteins, resulting in a non producing complex [1,3]. In arachidonic acid pathway, leukotriene B4 (LTB4) promoted skin inflammation by acting on peroxisome proliferator activated receptorα (PPARα). PPARα increased activity of activator protein (AP1) and NFkB led to recruitment of inflammatory cells. AP 1 inflammatory cascade led activation of matrix metalloproteinase which contributed to local tissue destruction and scar formation [29].

Retinoic replaced of disorganized dermal collagen fibers, caused an increased in epidermal thickness and returned to a more uniform size and electron density of the basal and spinous keratinocytes. For skin rejuvenation in photodamaged intrinsically and extrincally of the skin, at least 6 months of treatment is required. The predominance of one or other this effect depended on the concentration that be used.. Erythema and irritancy in higher concentrations might be produced. It was still reported hypertrophic scars and keloids to respond to a daily application of a 0,05% solution [3].

Oral isotretinoids reduced sebum production by reducing the size of sebaceous glands, in which the profoundly inhibited sebum excretion and affected comedo formation. Isotretinoin reduced 5α reductase activity in human liver and skin and reduced binding capacity of the androgen receptor in back skin, but the mechanism of action was unknown. Isotretinoin reduced the synthesis of DNA and the corporation of a lipid precursor 14 C acetate in human sebaceous gland in vitro. After oral administration, it appear to influence the endogenous vitamin A in skin by increasing the concentration of retinol, but decreasing that of its local metabolite, 3-dehydroretinol, a substance was known to accumulate in hyperproliferating tissue. All trans retinoic acid had no this effect, because it was more rapidly metabolized than isotretinoin [3].

In acne topical tretinoin was normally applied at the concentration of 0,025-0,5% in gel, lotion or cream. Stronger preparation had been tolerated in dark skinned races. After an initial exacerbation, it caused softening and expulsion of comedos in 3-4 weeks, and would prevent from these reforming if it was continued. Neither erythema nor peeling were necessary for this to be achieved [3].

Topical all trans retinoic acid decreased epidermal melanin without melanocyte loss, acted as depigmenting agents [1,3]. Tretinoin was down regulated TLR2 & TLR4, decreased secretion of pro inflammatory mediators and increased T reg differentiation in innate and adaptive immune response in the later stages of acne inflammation processes cause decreased of persistent inflammation [1,3].

Tretinoin was down regulate FGFR2 that caused regulation cell cycles controled and influenced cellular progression, cellular differentiation, cell survival and apoptosis, decreased differentiation and hyperproliferation of keratinocytes and sebocytes, decreased lipogenesis, caused smaller the sebocytes size, decreased sebum

production and alteration the composition of the cell surface lipid and attenuated growth and proliferation of infundibular keratinocytes [1,3].

Tretinoin was decrease TGFβ of Th 1 & T reg caused terminating keratinocytes & sebocytes activation cycles, decreased the expression of gen P53, increased growth inhibition, increased hair follicle regression [1,3]. Topical tretinoid unclogged pores by increased stickness of the keratinocytes inside follicle and decreased cell turnover, decreased sebum production, shrinked sebaceous glands and increased keratinocytes shedding [1,3].

Tretinoin blocked TLR2 activation directly, caused decreased primary cytokine IL1α (so decreased comedogenesis), decreased IL1β & TNFα (so decreased persistent inflammation by stopping positive loop in NFkB pathway). Tretinoin blocked TLR2 activation indirectly through NFkB pathway, caused decreased the production of secondary cytokine and loop of primary cytokines IL1β, TNFα and IL18 resulted decreasing of persistent inflammation [1,3].

Isotretinoin and other retinoid down regulated TLR2 expression on monocytes. Furthermore , retinoids induced Treg differentiation and down regulated Th17 differentiation and neutrophyl migration, but is was unknown whether this mechanism were modulated by isotretinoin in acne. Isotretinoin also down regulated the expression of MMPs -9 and -13, which played a central role in inflammatory matrix remodelling. Finally retinoids stimulated the released of GM-CSF, monocyte chemotactic protein-1 (MCP-1) and IL10 in cultured monocytes and macrophages [10,54].

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ALA PDT

5 aminolevulinic acid photodynamic therapy (ALA-PDT) was down regulated TLR2 and TLR4 receptor in the keratinocytes that examined by western blot assay, caused decreasing level of early inflammatory cytokines IL1α, TNFα, IL8 that examined by ELISA. So ALA-PDT could inhibit innate immune responses in keratinocytes in P acnes inflammation processes via down regulated TLRs pathways to supress early inflammartory cytokines IL1α, TNFα, IL8 [55].

UV induced IL10 production from keratinocytes lead to local and systemic immunity. Immunosupressive effects that occured after exposure to UV light exposure were the result of the liberation of keratinocytes derived IL10 into the systemic circulation that caused supression of T cell produced cytokines production. The absence of IL10 exaggerated irritant and contact sensitivity response [1].

Nitric Oxide

Nitric oxide Acted in NLRP3 pathway and decreased IL 1β

A potentially promising approach was the use of notric oxide (NO) releasing agent. NO had diverse functionally, including potent anti inflammatory, anti microbial and anti oxidant effects.

Friedman had shown that NO inhibited multiple elements of the inflammasome complex, including IL1β. There was developing nanoparticle that generate NO from a nitrit salt, slowly and safely releasing NO to kill P acnes and inhibited inflammation by acting on multiple component of the NLRP3 inflammasome.. This nanoparticles were ready for testing in clinic.

In Larry Weiss trials, the Nitrosomonas had been washed and wiped off on to super clean skin, would disrupt nitrogen cycle and leaving NO on the skin- mediated regulatory mechanism out of balance. Boston based biotech company AOBiome was taking a systems biology approach and testing a live suspension of Nitrosomonas eutropha, gram negative autotrophic bacteria that oxidazed ammonia and urea found on the human skin to nitrit and NO. The underlying premise was that ancestral commensal Nitrosomonas historically colonized the human skin as an essential component in our systemic nitrogen cycle, reducing inflammation, balancing the skin microbiome and helping to stabilize healthy skin. This bacteria had been depleted and eliminated from modern human skin because they were incredible sensitive to most soaps, surfactant, preservatives and fragrances found in cosmetics and hygiene products. Inadvertently, washed and wiped off people clean skin, would disrupt our nitrogen cycle and leaving our NO mediated regulatory mechanisms out of balance. By introducing Nitrosomonas to the skin, we could restore this NO balance without returning to life of hunter gatherer. AO Biome spray was currently in trials.

The activation TLR on macrophages by microbials ligands upregulated iNOS, which also result rapid generation of NO and powerfull microbial activity. Macrophages used this mechanism to contain some infectious organisms that were not susceptible to PMN attact such as mycobacteria, fungi, parasit and P acnes that had been resistant to phagocytosis. The stimulation of monocytes by activated T cells also led to generation of NO [1].

Short Chain Fatty Acid, Probiotic

SCFAs that produced by P Acnes when it fed on and broken down sebum, could leak out and triggered inflammation response from keratinocytes. But Richard Gallo, researcher from UCSD/University of California-San Diego, found that SCFAs/Short Chain Fatty Acid inhibited an enzyme in the nucleus of the cells that changed the way chromatin was folded. And in the skin cells it opened up the chromatin for inflammation. Inhibiting this action on the enzymes would stop the inflammation response.

Eric (Chun Ming) Huang, a dermatologist at UCSD's team worked on acne probiotic project. They had recently proven that the normal skin bacterium Staphylococcus epidermidis in live samples of skin of acne patients could ferment sugar such as glycerol to produce SCFAs that killed P acnes and reduce level of pro inflammatory cytokines. A molecular probiotic might be able to shape P acnes community, minimizing the ones that caused inflammation. A probiotic based

on this finding would be a novel, effective and safer modality for treatment acne.

Tetracycline, Doxicycline, Minocycline

Tetracycline, lymecycline and doxycycline were the antibiotics of the tetracycline group. Tetracycline were broad spectrum antibiotics, effective against various pathogens including ricketsiae, gram positive and gram negative bacteria. The antimicrobial properties of tetracyclines were known to reduce amount of propionibacteria and staphylococci on acne patient's skin. In vitro studies had shown that tetracyclines also had anti inflammatory effects that could have an important role in acne. They inhibited MMPs, scavenged ROS and had anti apoptotic effects. Tetracycline might also down regulated proinflammatory cytokines such as IL1β, TNFα, IL6 and chemokine such as IL8, MCP1, chemokine (C-C motif) ligand 3 (CCL3) and CCL4 and inhibited neutrophyl migration and degranulation [56-58].

Tetracyline, doxicycline, minocycline had anti inflammatory effect that was beneficial in the controle of inflammatory skin disorders in acne. They supressed IL8 production that was elicited by the activation of PAR2 gene/Protease Activated Receptor2 in NHEK/ Normal Human Epidermal Keratinocytes. Tetracycline was decreased the expression of PAR2 gene.42 Tetracyline attenuated the PAR2 - IL8 axis in NHEK and effectively modulated (decreased) pro inflammatory response in epidermal keratinocytes (skin), was a therapeutic target for the control of various cutaneous inflammation. In the NHEK the production of IL8 was stimulated by PAR2 gene. TNFα and IL1β that were induced production of IL8 was also supressed [42].

Tetracyclines were broad spectrum antibiotics with a tetracyclic naphtacene carboxamide ring. In addition to their acttivities as antibiotics, tetracycline showed a variety of biological actions: anti inflammatory activity, antiapoptosis activity, inhibition of proteolysis and supression of angiogenesis and tumor metastases. Clinically tetracycline had been used to treat rheumatoid arthritis and various skin disorders, such as inflammatory acne, rosacea, bullous dermatoses and neutrophilic dermatosis. The anti inflammatory activity of tetracycline included the modulation/inhibited of lymphocytes activation and neutrophyl chemotaxis, possibly by inhibiting/destruction of matrix metaloproteinases, phospholipase A2, nitric oxide synthetases and/or caspase1, inhibit TNFα and IL8 [42].

The ability of tetracycline to bind Ca 2+ and Mg 2+ accounted for some of those biological activities via the chellation of those cation and their transport into intracelluler compartment. The PAR2 mediated production of IL8 depended on the expression of the PAR2 gene, which was not reduced by the concentration of tetracycline used. The effect of the tetracycline in the production of IL8 were due to decreased expression of the PAR2 gene [42].

Pro inflammatory cytokines, matrix metalloproteinases and antimicrobial peptides were upregulated in the inflammatory stages of acne, which could be effectively treated with tetracyclines. In cutaneous inflammation, Tetracycline was reduced neutrophyl chemotaxis. Tetracycline modulated the PAR2-mediated production of IL8, which recruited neutrophyls to the inflammatory lessions. In rosacea skin, PAR2 was widely expressed by keratinocytes, in which endogenous proteases such as kallikrein-related peptidases that activated PAR2 gene were upregulated, could be treated by tetracycline, that had other mechanism involving angiogenesis in rosacea [42].

Friedman proved that tetracycline inhibited the activity of neutrophyls and dectructive enzym called metalloproteases. That both linked to acne inflammation and scarring. Tetracycline inhibited the expression and the activity of FGFR2b and down stream matrix metalloproteinnase [44].

Erytromycine, Roxitromycine, Clarytromycine

There were 14 membered ring macrolides erytromycine, roxitromycine, clarytromycine which had immunomodulatory activities, supressed/attenuated IL1β that induced production IL8 that was sinergized by the activation of PAR2. Erytromycine was P 450 inhibitor, interferred with FGFR2 signalling by its inhibitory effect on retinoid catabolism [44].

Down Regulation Fgfr2 Signalling [44]

antiandrogen (spironolocatone, cyproterone acetate, 5 avocuta)

Anti androgen drug worked at peripheral pilosebaceous androgen receptor and inhibited 5α reductase activity. Anti androgen supressed PGF ligand expression. Anti androgen affected FGF7/10-FGFR2-IL1α pathway, decreased FGF7 and FGFR2 that caused decreased IL1α, so it was failing to induce hyperproliferation and activation of infundibulum keratinocytes and sebocytes [44].

Androgen dependent FGFR2 mediated would release IL1α by infrainfundibular keratinocytes. IL1α acted as primary stimulus leading to hyperprolifferation of infundibulum keratinocytes and comedo formation, induced secretory keratinocytes activation inducing the perifollicular infiltration by T cell and macrophages and initiated immune response. The activation of IL1α receptor associate Kinase (TRAKs) that were involved in IL1 receptor or TLR mediated signal transduction process would regulate innate and adaptive immune response. FGFR2 activation led down stream the expression of IL1α that induced terminal differentiation of sebocytes, upregulated MCSR and MCR dependent lipogenesis [44].

Benzoil Peroxide

Benzoil peroxide induced FGFR2 downregulation by by lysosomal receptor degradation.

Tetracycline

Tetracycline inhibited the expression and the activity of FGFR2b and down stream matrix metalloproteinnase.

Retinoids

Retinoids attenuated the FGFR2 pathway at several regulatory levels of the signal transduction cascade critical for cycle cell control, cell proliferation, differentiation and lipogenesis.

Erytromycine

Erytromycine was P 450 inhibitor, interferred with FGFR2 signalling by its inhibitory effect on retinoid catabolism.

Inhibitor Of Ectopeptidase Dipeptidyl Peptidase Iv & Aminopeptidase

Inhibitor ectopeptidase suppressed proliferation and IL2 production of P Acnes that stimulated T cell to increase expression of TGFβ1 that activated T reg to secreted IL10 (down regulated cytokines). TGFβ deactivated signal that promoted reversal of activated keratinocytes,

so TGFβ terminating keratinocytes and sebocytes activation cycle in acne vulgaris. Ectopeptidase inhibitor induced IL1 receptor and decreased lipid production, supressed proliferation of sebocytes, increased terminal differentiation, supressed DNA synthesis of viabel SZ95 sebocytes.

Interleukine 1α and β Antagonist/Targeted Agents
Anakinra, rilonacept. Canakinumab

Anakinra was antagonist IL1 receptor, a recombinant form natural IL1 receptor antagonist. Rilonacept was soluble decoy IL1 receptor. Canakinumab was monoclonal IL1β antibody. They acted as antagonist once bound to its receptor, IL1 triggered cascade of inflammatory mediators, chemokines and other cytokines [59].

IL1 was a master cytokine of local and systemic inflammation. IL1 was highly active pro inflammatory cytokine that lower pain thresholds and damaged tissue. Monotherapy blocking IL1 activity in treated autoimmune diseases which acne clinical manifestation in rare genetic autoinflammatory disorder PAPA (pyogenic arthritis, pyoderma gangrenosum and acne) syndrome and a chronic inflammatory disorder syndrome SAPHO (synovitis, acne, pustulosis, hyperostosis, osteoitis) syndrome [59].

TNFα Inhibitor/Target Agents
Etanercept, Infiximab, Adalimumab

Etanercept, along with infliximab and adalimumab was TNFα antagonist. TNFα was a major cytokine of the immune system that was deregulated in autoimmune disorders, causing inflammation and variety of systemic effects. Inhibition of TNFα resulted in a decreased in inflammatory markers such as IL1 and IL 6, leading to reduction in endothelial permeability and leucocyte migration. This medication were known to have many side effect eg. Infusion reactions, cytopenia, risk for infection, heart failure [60].

Etanercept was unique, it was fully humanized soluble fussion protein consisting of TNFα receptor linked to Fc region of IgG1. Whereas infliximab and adalimumab were monoclonal antibody that targeted TNFα. Etanercept worked by acting as decoy receptor for TNFα, thereby preventing it from binding cell surface receptors and subsequently decreasing inflammation [67]. TNFα antagonists were potent biologics used to treat a variety of autoimmune disorders such as rheumatoid arthritis, ankylossing spondylitis, Chron disease, psoriasis and psoriasis arthritis. Only a few cases of acne vulgaris had been associated with the use of this biologic agents, particularly infliximab and adalimumab. There were reported the use of etanercept in treating severe acne and acne conglobata [60]. But that was reported a rare case of etanercept induced cystic acne associated with psoriasis treatment. Many drugs had been implicated in acneiform eruptions mimicking acne vulgaris and TNFα antagonists (infiximab and adalimumab) were reported to have acneiform eruption side effect when using this drugs to treat psoriasis [60].

Theoritically anti TNFα agents should supress acne rather than induce it due to their inhibition of the inflammatory markers TNFα, IL1α and IFNɣ, which thought to play a role in hypercornification of the infundibulum. Thus the mechanism of TNFα antagonists associated with acneiform eruption remained unkown and need further research [60].

New clinical drugs targeted the TNF pathway, a humanized anti TNFα antibody infliximab (remicade) and soluble TNF receptor etanercept (enbrell), that appeared to have more profound effect on psoriasis that other autoimmune disease [1].

Leukotrien B4 Antagonist
Zileuton, Montelukast, Zafirlukast

Leukotriene (LTB4) was a family of eicosanoid inflammatory mediators produced in leukocytes by the oxidation of arachidonic acid (AA) and the essential fatty acideicosapentaeoic acid (EPA) by the enzym arachidonate 5 lipooxigenase. The inflammatory chemical leukotriene B4/LTB4 was known to upregulate sebum production. Synthetic inhibition of LTB4 , in the form of drug zileuton led to significant improvement in acne. Eicosapentaeoic acid (EPA) from fish oil and gamma linoleic acid (GLA) from borage oil have reported to inhibit the conversion AA to LTB4 to the same degrees as the LTB4 inhibiting acne drug candidate zileuton.

Epigalocatechin 3 gallate (EGCG), a polyphenol from green tea had also been suggested to be helpful in acne due to its well documented anti inflammatory and anti oxidant activity. EGCG influenced hormonal aspects of acne since it was known to posses 5 α reductase inhibiting property for topically applied.

Zafirlukast and montelukast were antagonist LTB4 receptor, were effective-safe and recomended to treat moderate levels of acne vulgaris [61].

The Increasing of 5 lipooxygenase enzym that responsible in arachidonic acid pathway- leukotriene B4 (LTB4) and then promoted skin inflammation by acting on peroxisome proliferator activated receptorα (PPARα). PPARα increased activity of activator protein (AP1) and NFkB led to recruitment of inflammatory cells. AP 1 inflammatory cascade led activation of matrix metalloproteinase which contributed to local tissue destruction and scar formation [29]. Leukotrien B4 was a family of eicosanoid inflammatory mediators produced in leukocytes by the oxidation of arachidonic acid (AA) and the essential fatty acideicosapentaeoic acid (EPA) by the enzym arachidonate 5 lipooxigenase. The inflammatory chemical leukotriene B4/LTB4 was known to upregulate sebum production [52].

Steroids also blocked arachidonic acid pathway, that caused decreased of leukotriene B4 (LTB4) that promoted skin inflammation. Topical steroid avoided the possibility of hypertrophic scar and keloid [3].

Immunotherapy

In study was reported the elevated IgE levels that related with clinical severity in a group, but another group was found no changed in total IgE levels [3]. Female had better defence mechanism than male against P acnes [3]. Most acne patient had no misfit immunological reaction [3]. Skin testing with heat killed suspensies of *P acnes* demonstrated that subject with severe acne produced a greater inflammatory reaction at 48 h than other subject, suggested that host response might been important [3]. Changing in neutrophyl chemotaxis might been the secondary event [3]. *P acnes* polypeptide were detected in serum of the acne patients but were not in normal individu [3]. Acne fulminant showed exaggerated delayed hypersensitivity reaction to *P acnes* [3].

Benefits of immunization as immunotherapy based approaches, which represented a solution for limitting the development of

antibiotic resistant P.acnes. Various immunization based approaches had been developed over last decades, including heat killed pathogen based vaccines, virulence factor spesific antibodies, vaccination against cell wall-anchored sialidase, monoclonal antibodies to to the Christie-Atkins-Munch Peterson factor of P.acnes, anti toll like receptor vaccines and natural antimicrobial peptides. Immunotherapy showed efficacy for acne, was a realistic treatment option for the future and need further researces [62].

Huang team from UCSD also worked on a vaccine to control the growth of P acnes. The reseachers had discovered that blocking CAMP/Christie Atkins Munch Peterson factor secreted P acnes prevented inflammation caused by P acnes in human skin. Using secretory CAMP factor as an antigen, the vaccine (monoclonal antibody) would neutralized it , reducing inflammation without killing P acnes, which was harmless on healthy skin. This approach had the potential result in long term cure of the disease.

The pathways of TLR activation meant to adjuvant immune responses in vaccines and treatment for the infectious diseases [1].

Chapter II
Case Report

5 cases of acne vulgaris 3rd to 4th grade in female, 20-30 years old that had been treated by combination of topical tretinoin 0,05% and flucinolone acetonide 0,025% for 4 weeks were reported. The result were good, there were decreasing of acne grading severity based on Cunliffe acne grading severity classification, from 3rd to 4th grade acnes to 1st grade acnes or no acne. No seriously side effects were reported. Tretinoin 0,025% was choosen as maintain treatment.

Chapter III
Discussion
The Role of Pro Inflammatory Mediators in Acne

In acne vulgaris, the released of pro inflammatory mediators/ biological active mediators like cytokines, chemokines, leukotriene, AMPs, enzymes (protease, lysozim) that triggered by P acnes unlikely to have protective role, but it had harmfull effect in acne by promoting persistent inflammation, tissue destruction, scarring and pigmentation. TLR2, NLRP3, PAR2 gene, FGFR2 were a logical target for therapeutic intervention to block Pro inflammatory mediators like cytokines responses in acne and other inflammatory conditions in which tissue injury was detrimental to the host. Many acne drugs that acted in supressing or blocking of pro inflammatory production and acted as anti immunological reaction were discussed as novel treatment in acne vulgaris chronic inflammation processes.

The immune system had to protect the skin against harmful microbial, chemical and physical insults. The activation of innate immunity provided the first rapid but non spesific response against those harmful attacts. The activation of adaptive immune system was more spesific due to immunologic memory. However, there was the activation of both innate and adaptive immunity in very early event in the formation of acne lessions [10-12].

But in acne lession the immune response was different. There were adaptive immune response to non spesific antigen that be involved in early stage of acne inflammation processes (less than first 6 hours). It could be found CD4+ Tlymphocytes in early 6 hours comedos formation. Then developed both innate imunity (acted by Phagocytes, Human Beta defensin 1,2 of keratinocytes and Complement) and adaptive immunity (Acted by Th1, Th2, Th17, Treg and B cell differentiation) to spesific antigen P acnes that later could be recognized and involved in later stages of acne inflammation (more than 6 hours of lession).

Both non spesific (S epidermidis, P Ovale) and spesific antigen (P acnes) were involved in complement classic and alternative pathway in acne inflammation process, and it could be happened in both early and later stages of acne inflammation processes. Non spesific and spesific antigen were bound to antibody than could be attached by complement to be opsonized, than phagocytes by polymorphonuclear cells [1,3,4].

Innate Immunity

In acne vulgaris inflammation processes, innate immunity was a defense mechanisms that were use by the host immediately after encountering a foreign ligand. These included the physical bariers such as the skin and mucosal epithelium, soluble factor such as complement, antimicrobial peptides, chemokines and cytokines and cells of the innate immune system including monocyte/macrophages, dendritic cells and polymorphonuclear leukocytes (PMNs) [1]. Innate immune responses in acne were mediated by a variety of different cell types including monocytes, macrophages, neutrophyls and dendritic cells as well as non immune cells such as keratinocytes and sebocytes [10,13,16].

The inflammation process in patients with acne was supported by polymorphonuclear neutrophyls, which produced a large number of free radicals by prostaglandins, leukotriene B4 and complement [17].

Physical barier (innate immunity) play a role acne inflammation were:

1. Skin (stratum corneum), a terminal differentiation of keratinocytes was protecting from external environment.
2. The free fatty acids created an acidic environment that inhibited collonization of some bacteria, but it flourished P Ovale and P acnes colonization in acne vulgaris patients [1]. FFAs, squalene and wax esters had recently been identified as autoantigens presented by CD1a of langerhans cells and recognized by autoreactive T cells that later played a role in adaptive immunity.

Keratinocytes played a role in innate immunity, they were stimulated by injury or stimulation with exogenous factors such as lipopolysacharides (LPS), silica, poison ivy cathecols, Staphylococcus toxins and UV radiation. and produced Antimicrobial peptides (human β defensin 1,2,3 and cathelicidins). In normal condition it secreted very low level of proinflammatory cytokines. High level released of cytokines (IL1, IL6, IL8. IL10. TNFα) were secreted by keratinocytes in injury factor stimulation. These cytokines induced differentiation and growth of the keratinocytes or other resident, induced migrating cell in the epidermis-dermis and vessels, acted as important mediators for local and systemic inflammatory and immune response [1].

Phagocytes such as macrophages and PMNs were the major component of innate immune system and had capacity to detect

the microbial pattern using complement receptor on their surface. PMNs also phagocyted microbes coated with antibody that be opspnized by complement active and alternative pathways. PMNs also had spesific receptor that could recognized pathogens directly. Phagocytes expressed pattern recognition receptors (PPRs) that fucntion to be recognized pathogen asscociated mollecular patterns (PAMPs). Examples of the PPRs included mannose receptor that recognized mannan (yeast), CD14 that recognized Lipid A portion of LPS, surface Toll Like Receptors (TLRs) that recognized lipopolysacharides or peptidoglycans of the bacteria. PPRs were nonclonal receptor that were present on all phagocytic cells e.g. macrophages, keratinocytes, sebocytes and did not dependent on immunologic memory because they were a germ line encoded (component of innate imunity) [1].

Complement was a part of plasma membrane. There were 2 complement activation: classical pathway (that was mediated by immune complex) and altenative pathway (that was mediated by yeast or bacterial cells) [30]. There was complement activation that involved in the early to later stages of inflammation and P acnes were capable for triggering both the alternative and classical complement pathways.

Comedonal contents and P acnes had been shown to activate complement via both classic and alternative pathways. Complement-3 (C3) immunoreactivity had been detected in early stage inflammatory in acne lessions in the walls of small dermal blood vessels and the dermo epidermal junction. In the late stage inflammatory lessions C3 deposition was much less prominent [10]. Complement activation caused lysis bacteria and virus, opsonization, inflammation [28].

P ovale (through an alternative pathway of complement activation) activated cellular immune response and humoral immune response [28]. Complement was the part of immune system that enhanced the ability of antibodies and phagocytic cells to clear microbes and damage cells from an organism, promoted inflammation and attacked the pathogen plasma membrane. It was part of innate immune system (which was not adaptable and did not change over the course of individual`s lifetime) and it could been recruitted by the adaptive immune system to finish the action [3,4].

P acnes antigens activated the complement system and provided migration of free radicals, neutrophyls and macrophages with the following production of proteolytic enzymes, IL1α. IL 1β, IL8 and TNFɣ, which caused inflammation and complex cascade of pathogenetic mechanisms of the disease. The role of cellular and humoral immunity and cytokine activity in acne was still a subject of study [18].

Biological active mediators that played a role in innate immunity were produced by:

1. Binding of the PAMPs bacterial's to innate surface PPRs (TLR2 & TLR4) of the keratinocytes, sebocytes, innate cells-monocy tes,macrophages,neutrophyls,mast cells, NK (produced IL1α, TNFα, IL1β, IL2, IL4, IL6, IL8, IL10, IL12, IL1β, IL18, IFNγ),
2. Bacterials activation to innate cell cytoplasm PPRs NLRP3 inflammasome of sebocytes (produces IL1β, IL18-IFNγ, IL33,IL6,IL8)
3. PAR2 gene. activation of the sebocytes & keratinocytes by proteases that were produced by P acnes, caused deffect of differentiation, enhanced lipogenesis & SREBP1 expression, induced IL8-TNFα-HBD1,2-ICAM that caused inflammation, produced matrix metalloproteinase MMP1-1-2-3-9-13 that degraded colagen & caused scarring, increased of melanin intake/melanin transfered that caused pigmentation, Increased ICAM1 of endothelial cells that roled in inflammation and wound healing, induced itching generation due to mast cell triptase, and disturbed epidermal barier homeostasis via the supressed secretion of lipid lamelae of keratinocytes.
4. Activation of FGFR2 of the keratinocytes that stimulated by IL1α. IL1α increased producing by androgen stimulation. FGFR2t caused cell cycles control disturbance, terminating differentiatiation and proliferation, hyperkeratinization and comedo formation, sebaceous gland hypertrophy-proliferation & differentiation, increased of lipogenesis, important for skin appendages formation and pilosebaceous follicle hemostasis,
5. Leukotriene (LTB4) was biologically active mediators,a family of eicosanoid inflammatory mediators that were produced in leukocytes by the oxidation of arachidonic acid (AA) caused inflammation, and activated AP1 cascade to produce matrix metalloproteinnase that caused soft tissue destruction and scarring.

Adaptive Immunity

Adaptive immunity in acne vulgaris was caused by non spesific antigen that was involved in early stage of inflammation (less than 6 hours), but in the later stages (more than 6-72 hours) adaptive imunity to spesific antigen then developed too following innate imunity to spesific antigen reaction.

The adaptive immune response was more complex than the innate. The antigen first should be processed and recognized. Once the antigen had been recognized, the adaptive immune system created an army of immune cells specifically designed to attack that antigen. Antigen spesific T and B lymphocytes mediated adaptive immune responses. Activated B cells secreted immunoglobulins and were involved in humoral immunity, whereas T cells were involved in cell mediated immune response [45,26].

T cells were divided into two main groups, CD4+ helper T cells (Th) and CD8+ cytotoxic T cells (Tc) and they identified antigen-derived peptides by their antigen receptor. Th cells recognized antigens bound to MHC class II molecules expressed on APCs. Recognition of foreign antigen-derived peptides by naive T cells led to a process that included massive proliferation and differentiation into distinc T cell subsets according to the corresponding cytokine profile. Th cells helped B cells to produce antibodies, activated Tc cells, and recruited and activated other immune cells. Tc cells acted as killer cells, but like CD4+ cells, they could also exert regulatory functions and produced different cytokines [10].

Th cells were the central players in adaptive immune responses. Many types of specialized Th cells had been identified. The main Th cell lineages were Th1, Th2, Th17 and regulatory T (Treg) cells. Th1 cells promoted cell mediated immunity by activating macrophages and CD8+ T cells to kill viruses and other intracellular pathogens. Th1 cells also contributed to the pathogenesis of autoimmune diseases. Th2 responses were critical for IgE production and activation of eosinophyls, mast cells and basophyl. Th2 were important for the elimination of helminthic parasites but now they were known better for their role in the pathogenesis of astma and other related allergic conditions. Th17 cells recruited and activated neutrophyls

and contributed to their host defence against extracelluler bacteria and fungi. They contributed to chronic inflammation associated with many inflammatory and autoimmune disorders. Treg cells were immunosupresive and maintained self tolerance, prevented autoimmunity and controlled immune responses during infection and cancer. In addition to four major lineages, other potential new Th lineages had been proposed including Th3, Th9, Th22, follicular Th (Tfh) cells and type 1 regulatory (Tr1) cells. Since the signature cytokines produced by these cells were also the products of Th1/Th2/Th17/Treg cells and the trascription factors, they express were not unique, it was possible that these cells represent subsets of the four main lineages [11,46].

Adaptive immunity in acne vulgaris inflammation were roled by T lymphocytes (activation and differentiation of naive T cell to Th1, Th2, Treg, Th17) and B cell.

1. Bacteria was recognized by Antigen presenting cells-macrophages (through MHC class II of phagocyte innate cell immunity) that bound to the CD4+ of naive T cell lymphocytes, than T cell differentiated to Th1(produced IFNγ, IL18, IL2), Th2 (produced IL4, IL5, IL13), Treg (produced IL10, TGFβ), Th17 (produced IL17 α&β , IL21, IL22, CCL20).
2. B cell differentiation produced immunoglobulin, that was activated by Th2.

Stage of Acne Inflammation Process

In the early non inflammed and inflammed lession had shown that there were activation of the classical and alternative pathways [3]. And there were the type 4 immunological reaction to a non spesific antigens in the prior of obvious duct ruptured [3].

In the later, in the moderate and severe inflammation , there was disruption of the duct and macrophage giant cell foreign body reaction [3]. An amplification phase via antigen dependent T cell responses to other comedonal components for example *P acnes*, might then developed [4]. The intensity and duration of the subsequent cell mediated response would been depended on many factors, including the degree of individual sensitization to their cutaneous microflora [4]. Following the disruption of cell wall, neutrophyl would been attracted into the duct by microbial chemotactic factor, that was proved by a study that demonstrated the capability of *P acnes* to attract neutrophyl in vitro [4]. So *P acnes* might caused inflammation because this organism had been shown to secrete chemotactic factors and the chemotactic activity had been shown in comedones [1]. Low mollecular weight chemotactic factor did not require serum complement for activation and because of its small size, it could probably escaped from follicle and attracted polymorphonuclear leukocytes [1]. If polymorphonuclear leukocytes enter the follicle, they could ingested *P acnes* organisms, resulted the release of hydrolytic anzymes which in turn, might been importance in producing follicular epithelial damage [1]. Polymorphonuclear leukocyte ingested *P acnes* was anti *P acnes* dependent antibody (ADCC) [1].

In the late phase of inflammation, *P acnes* dependent T cell lymphocyte response could been found, there were variations in Cell Mediated Immune Response depended on individual microflora sensitization [1,4]. Circulating antibody to *P acnes* were elevated in patient with severe acne [1].

How Did Biological Active Mediators Work?

Cytokines function was for differentiation and growth of keratinocytes, induced migrating of inflammation cell in the dermis and epidermis, acted as important mediator for local & systemic inflammation and immune response.

- ✓ IL1α played a role in stimulation of abnormal keratinocyte proliferation, differentiation and hypercornification that revealed obstructions, follicles impaction and distention that formed comedos [2,3,13]. IL1α was an important inducer of keratinocytes proliferation, hyperkeratinization and reduced desquamation of comedo formation by stimulated fibroblast to secrete FGF7 which stimulated FGFR2b to mediate keratinocytes proliferation, disturbance of cell cycle control, termination of differentiation, hyperkeratinization and comedo formation, sebaceous glands atrophy and lipogenesis [1,3]. IL1α was low activated by lymphocytes, chemotaxis, activation of neutrophyl than induced inflammation [28].
- ✓ TNFα increased langerhans cells migration to limphonodes to be matured. TNFα was an important mediator of cutaneous inflammation and its expression was induced in the course of almost all inflammatory responses in the skin. Normal human keratinocytes (NHEK) and keratinocytes cell line produced substantial amount of TNFα after stimulation with LPS, UV light and irritant substance and contact sensitizers [1].
  Langerhans cell was the spesific innate immune cell in the epidermis, LC took up pathogen after encountering by endocytotic process and migrated to the daining lymphnodes where they developed to mature dendritic cell, and this process was stimulated by TNFα. Those migrating cells losed the ability to take up and process antigen but they upregulated MHC molecules and costimulatory mollecules to activate innate T cells. T.
- ✓ IL1β secreted partially via NLRP3 mediated pathway. When phagocytes were stimulated by live P Acnes, caspase 1 was upregulated induced IL1β secretion in NLRP1 and NLRP3 mediated pathway, was an innate immune response, that caused inflammation in acne pathogenesis [22]. IL1β had been shown as a potent inducer of pro inflammatory cytokines IL6, IL8 in sebocytes suggesting the potential role in diseases of pilosebaceous unit such as acne [22].
- ✓ IL1β and TNFα played a role in persistent inflammation. IL1 and TNF induced cell adhesion molecule expression on the endothelial cells (both selectine and imunoglobulin super family members such as intercelluler adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). Induced a variety of cells to produce a host of additional cytokines and induced expression of chemokines that provided chemotactic gradient, allowing directed migration of spesific leukocytes subsets into the site of inflammation [1].
- ✓ IL6 was the important cytokine for the skin and was subject to dysregulation in several human skin manifestation diseases. IL6 was produced in a regulated fashion by keratinocytes, fibroblast and vascular endothelial cells, as well as by leukocytes infiltrating the skin. IL6 stimulated the proliferation of human keratinocytes under some condition, and the expression was increased in psoriasis and acne vulgaris [1-3]. IL6 interacted with a hemopoitin receptor family member gp130 (glkycoprotein 130 kDa).
- ✓ Chemokines IL8 played a role as inducer of chemotactic factors might played an important role in attracted neutrophyl to the

pilosebaceous unit that led to release lysosomal enzime that led to rupture follicular epithellium and further inflammation [17]. Furthermore *P acnes* released lipases, proteases, hyaluronidase which contributed to tissue injury [17]. IL 8 induced chemotaxis and activation of neutrophyl and T cells [17,28].

Neutrophyls were normally not present in the skin, however during inflammatory process, after stimulated by IL8 (a chemokines that had low mollecular weight that had chemotaxis effect), these cells migrated to the sites of infection and inflammation where they were the earliest phagocytic cells to be recruited, than neutrophyls degraded after pathogen (P acnes) phacytosis that was caused by lysosym enzymes that were produced [1].

✓ Cytokine IL12 played a role in innate immunity as stimulator for NK cell proliferation and citotoxicity and induced/activated Th1 in adaptive immunity. IL12 promoted development of Th1 mediated immune response. And overproduction of Th1 cytokine such as IL 12 was implicated in the development of tissue injury in a certain autoimmune and inflammatory disease [17]. IL12 had active form a heterodimer of two protein p35 and p40. IL12 was produced by Antigen presenting cells as dendritic cells, monocytes and macrophages as well as certain B cells in response to bacterial component, GM-CSF and IFNγ. Activated keratinocytes was the additional source of IL12 in skin. Human keratinocytes constitutively made the p35 subunit and the expression of p40 subunit could be induced by stimuli such as contact allergens, phorbol esthers and UV radiation. IL12 was immunoregulatory cytokine for initiation and maintenance Th1 response. Th1 responses was depend on IL12, provided protective immunity to intracellular bacterial pathogen and mediated Th1 response in autoimmune disease. IL12 had simulatory effects on NK cells, promoting their proliferation, cytotoxic function, and the production of cytokine including IFNγ and stimulated protective antitumor immunity [1].

✓ IL18 (that innitialy described as IFNɣ inducing factor) acted directly to Natural killer cells to stimulate IFNɣ synthesis and upregulated their killing capacity [18,25]. It was believe that IL18 derived from keratinocytes might be than involved in cutaneous Th1 type adaptive immune response [25]. IL18 induced Th1 in adaptive immunity and induced Natural killer to produced IFNɣ. IL18 initially described as IFNɣ inducing factor, was a recently characterized cytokine that shared structural features with IL1 family of proteins. IL 18 induced IL8 gene expression and synthesis [25].

✓ The most prominent function of IL17 was neutrophyl chemotaxis mediated by the production of granulocyte colony stimulating factor (G-CSF) and chemokines such as IL8/Cxcl8. IL17 also induced chemo attractans eg. Ccl20, Gm CSF for lymphocutes, DCs and monocytes and modulate the production of AMPs. Importantly IL17 synergized with other inflammatory cytokines in particular TNFα, IL1β, IL22 and IFNɣ [10]. In addition of its potential function to attract the neutrophyls in early phases of acne inflammation, IL17 accounted for the increased expression of AMPs/anti Microbial Peptides in acne lession. IL17 together with IL1 and TNFα induced the production of several MMPs/Matrix Metalloproteinnases, whose expression was also increased in acne [10].

There was activation of the IL17/Th17 axis, which may together with IL1 be an essensial component in the immunopathogenesis of acne. The IL17/Th17 axis was activated in clinically early stage acne lessions. The inflammatory infiltrates contained the CD4+ that were Th1, Th17 and Tregs, and also CD68+ macrophages, mature CD83+ DCs cells and CD8+ T cells [10]. As well as by CD4+ cells and neutrophyls, IL17 could also be produced by several other types including Tc cells, group 3 innate lymphoid (ILC3) cells, gama teta cells, NK cells, Mast cells and macrophages. Keratinocytes were principal cellular target of IL17A, but IL17 receptors were constitutively expressed on fibroblasts, osteoblasts, chondrocytes, macrophages, DCs and endothelial cells. It was noticed that IL17 expressing cells were mainly lymphocytes with minority of neutrophyls and mast cells [10].

The expression levels of TGFβ and FOXP3 m RNA were lower in acne patients uninvolved skin than healthy normal skin [10]. P.acnes induced IL17 expression in human peripheral blood mononuclear cells (PBMC), that induced naive CD4+ T lymphocytes to differentite/expressing into Th 17 cells and Th1 cells expressing IFNɣ, IL1α, IL6 and TGFβ/transforming growth factor regulated P. Acnes and induced IL17 response as they did in other system. Finding IL17 and Th17 cells proved the possibility that acne was not uniquely a Th1 mediated disease, suggest that immune acne immune responses were more complex than thought previously. In addition showing that P. Acnes induced the production of IFNɣ/IL18 , a key cytokine marker for Th1 cells, it was demonstrated that P.acnes modulated IL17 and IL22 production as well as the expression of Th1 receptors on PMBCs [29].

✓ IL4, IL10 played a role as down regulation/feed back, acted as TLRs antagonists. IL10 was one of several cytokine some produced by Th2 cells that inhibited cytokine production after activation of T cells by antigens and APC, primarily exert regulatory rather than stimulatory effects on immune response. IL10 acted through the surface receptor on macrophages, dendritic cells, neutrophyls, B cells, T cells and NK cells. Inhibitory effect of IL10 on APC such as monocytes, macrophages and dendritic cells included inhibition of expression of class II MHC and costimulatory molecules (e.g. B7-1, B7-2) and decreased production of T cell stimulating cytokines (e.g. IL1, IL6 and IL12). But IL10 stimulated (rather than inhibited) B cell synthesis of class II MHC molecules and costimulates the growth of mast cell. The ligand binding chain of the receptor was homolog to the receptor for IFN α.β & ɣ and signaling events mediated through the IL10 receptor use Jak STAT pathway [1].

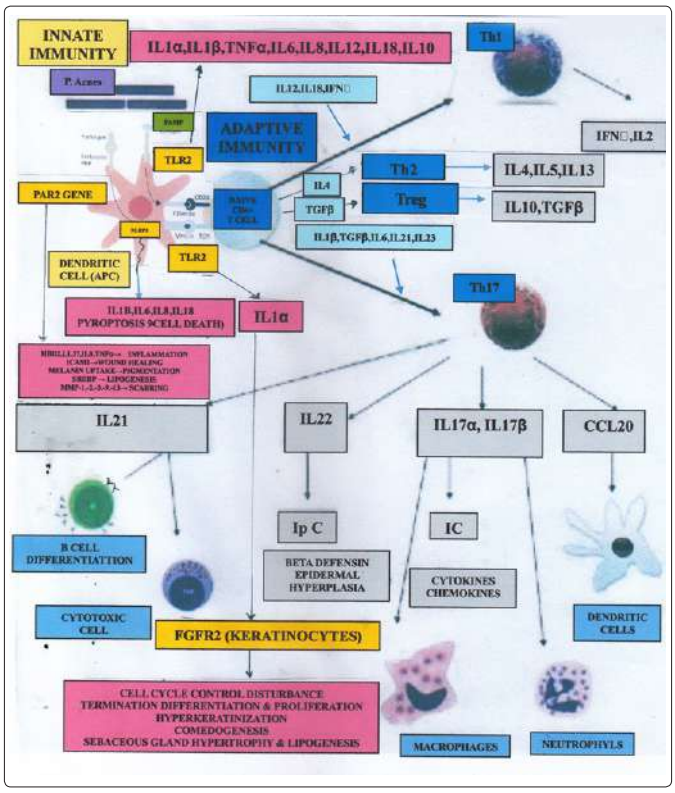
✓ Epidermal keratinocytes was the major source of IL10 in skin. IL10 production by keratinocytes was upregulated after cell activation and one of the stimuli for activation was UV irradiation. UV induced keratinocytes production lead to local and systemic immunity. Immunosupressive effects that occured after exposure to UV light exposure were the result of the liberation of keratinocytes derived IL10 into the systemic circulation. The absence of IL10 exaggerated irritant and contact sensitivity response [1].

✓ Leukotriene(LTB4) roled in soft tissue destruction and scar formation [3,4]. LTB4 was eicosanoid, a biologic active mediators that function as hormones. LTB4 was sinthesized from arachidonic acid. Arachidonic acid was stored in the phospholipids of cell membranes and was released by phospholipases after the spesific stimulation of cell surface receptors.They was careful regulated released by receptor stimulation, where arachidonic acid was released during membrane dissolution induced by cellular necrosis or apoptosis.

Once cleaved from the membrane, arachidonic acid was immediately oxygenated to form products that were released from the cell or were esterified into membrane phospholipids [1]. Leukotrienes were synthesized predominantly by 5-lipooxygenase enzymes that were found in leucocytes, macrophages, mast cells, keratinocytes and lung epithelium, that metabolized arachidonic acid to form leukotriene intermediate (LTA4) that was hydroxylazed by LTA4 hydrolase (a cytosolic enzyme that was found in leukocyte, lung, heart and intestine) to form LTB4 and LTC4. Leukotrienes had individual export carier system, that was ATP dependent, than leukotriene released from the cells [1].

LTB4 was predominant eicosanoid metabolit of neutrophyls that also produced by macrophages. LTB4 was most potent chemotactic and chemokinetic substance, could induce degranulation of polymorphonuclear leukocytes and their adherence to vascular endothelium.

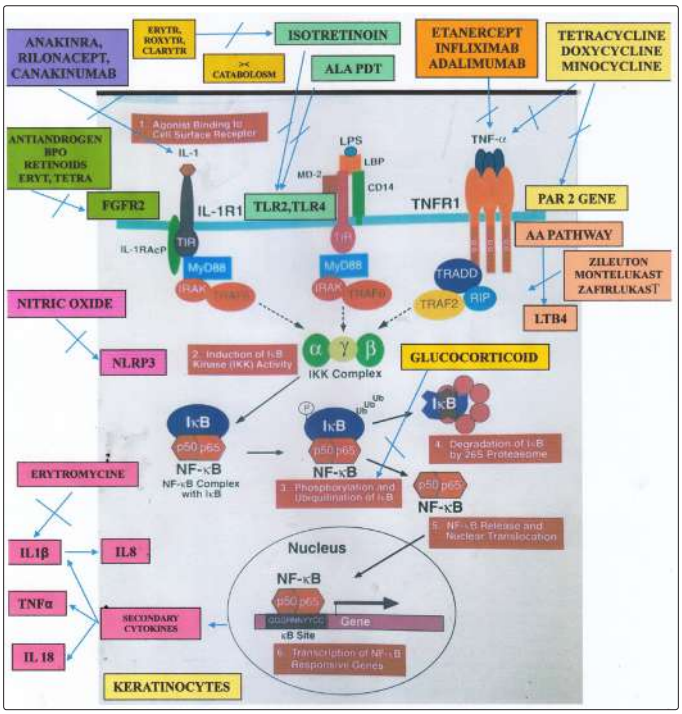
✓ Matrix metalloproteinases roled in inducing acne vulgaris inflammation and soft tissue destruction, collagen degradation and scar formation. The upregulated MMPs might contribute to acne pathogenesis by inducing inflammation and tissue destruction. Increased MMP activity produced greater amount of degraded collagen in the lessional acne skin. Matrix breakdown was followed by imperfect repair, which was thought to result in acne scarring. MMPs were a family of zinc dependent endopeptidases, which degraded extracellular matrix components under both normal and inflammatory conditions and several MMPs including MMP-1,-2,-3 and-9 were up regulated in acne lessions [35,37].



Application In Dermatology Practice

In acne vulgaris, the released of pro inflammatory mediators like cytokines that triggered by P acnes unlikely to have protective role, but it had harmfull effect in acne by promoting inflammation and tissue destruction. TLR2, NLRP3, PAR2 gene, FGFR2, blocking NFkB pathways, blocking/antagonists biologically active mediators were a logical target for therapeutic intervention to block the working of Pro inflammatory mediators like cytokines, chemokines, leukotriene responses in acne and other inflammatory conditions in which tissue injury was detrimental to the host, an acted as anti immunological reaction drugs.

Some anti immunological reaction drugs that had been reported were (the effectivity of some drugs were still in research) : Acted in NFkB central pathways (steroids), Antagonist TLR2 (Isotretinoin), Acted in NLRP3-↓IL1β (Nitric Oxide), Decreased of expression of PAR2 gene-↓IL8-↓TNFα-↓neutrophyl activation- destruction of enzym metalloproteinnase & as TLR2 antagonist (tetracyline, doxicycline, minocycline, erytromisin, roxytromycine, clarytromycin), down regulated TLR2 & TLR4 receptors-↓ IL1α, TNFα, IL8(ALA PDT), antagonist FGFR2 (anti androgen, isotretinoin, BPO, tetracycline, erytromycine), IL1 targetted agents (Anakinra, rilonacept, canakinumab), TNFα inhibitor (etanercept, infliximab, adalimumab), Leukotrien B4 antagonist (zileuron), Leukotrien antagonist (montelucast), Immunotherapy (immunization-CAMPS was neutralized & prevented inflammation, solved antibiotic resistance problems) [1,3,4].



This case report choosed topical tretinoin combined with topical steroid as treatment of choices for chronic inflammation in acne. This drugs were common, easy to get, cheap and safe enough for application in combination for short term periods. The combination had good result, fasten the healing of acne chronic inflammation processes. They acted as pro inflammatory antagonists drugs or anti immunological reaction.

Steroid acted in NFkB pathway that finally decreased IL1β & TNFα so decreased persistent inflammation, steroid upregulated TLR on macrophages by microbials ligands upregulated iNOS, which resulted rapid generation of NO (caused decreasing of IL1β) and powerfull microbial activity. Steroid blocked the production

of all arachidonic acid derivates (eicosanoids) through induction the production of lipocortin. Blocking arachidonic acid pathway caused ↓leukotriene B4 (LTB4) → ↓peroxisome proliferator activated receptorα (PPARα)→↓activity of activator protein (AP1) inflammatory cascade →↓matrix metalloproteinase → ↓local soft tissue destruction and scar formation.

Tretinoin acted as antagonist TLR2&TLR4, caused decreased of inflammation (decreased IL1β & TNFα), Decreased comedogenesis (decreased IL1α), Increased TGFβ secreted from T reg and attenuated FGFR2 pathway that caused terminating keratinocytes & sebocytes activation cycles, decreased the expression of P53 gen (anti keloids), ↑growth inhibition,↑ hair follicle regression. Downstream FGFR2 signalling primary deactivated signal of activated sebocytes and keratinocytes [46]. Tretinoin normalized differentiation and keratinization keratinocytes & sebocytes, prevented keratin plug formation, ↓cell turnover, ↑keratinocytes shedding Tretinoin decreased sebum production (shrink sebaceous glands) & comedo formation. Because of down regulated FGFR2 that influenced regulation of cell cycles control. Isotretinoin reduced 5α reductase activity in human liver and skin and reduced binding capacity of the androgen receptor. Tretinoin replaced disorganized dermal collagen fibers, caused an increased in epidermal thickness Tretinoin decreased epidermal melanin without melanocyte loss, acted as depigmenting agents.

Topical combination (steroid & tretinoin) had good result. Tretinoin decreased comedo, sebum production & hyperkeratinization, normalized keratinization and differentiation, replaced disorganized collagen fibers & formed thicker-elastic epidermis (skin rejuvenation), supressed the expression of P53 gen so decreased scar/keloid formation, decreased melanin transfer & pigmentation. Steroid decreased chronic persistent inflammation & scar formation and decreased erythema & irritancy that were caused by tretinoin [1,3,4].

Topical Tretinoin mechanism of action [1,3]:

1. Antiinflammatory, immunosupresive, anti mitogenic.
2. ↓erythema (produced transient vasoconstriction) and irritacy that could be caused by topical tretinoin.
3. Upregulated TLR2 receptor on macrophages by microbials ligands upregulated iNOS, which resulted rapid generation of NO (caused decreasing of IL1β) and powerfull microbial activity.
4. Stopped central NFKB pathway that caused blocking of primary cytokine positive loop activation (IL1β ,TNF α) & secondary cytokine production (IL18), caused decreased of persistent inflammation.
5. ↓ local tissue destruction and scar formation.

→ Block the production of all arachidonic acid derivates (eicosanoids) through induction the production of lipocortin.
→ >< arachidonic acid pathway → ↓leukotriene B4 (LTB4) → ↓peroxisome proliferator activated receptorα
(PPARα)→↓activity of activator protein (AP1) inflammatory cascade → ↓matrix metalloproteinase → ↓local soft tissue destruction and scar formation.

Topical Steroid mechanism of action [1,3]:

1. Antiinflammatory, immunosupresive, anti mitogenic.
2. ↓erythema (produced transient vasoconstriction) and irritacy that could be caused by topical tretinoin.
3. Upregulated TLR2 receptor on macrophages by microbials ligands upregulated iNOS, which resulted rapid generation of NO (caused decreasing of IL1β) and powerfull microbial activity.
4. Stopped central NFKB pathway that caused blocking of primary cytokine positive loop activation (IL1β ,TNF α) & secondary cytokine production (IL18), caused decreased of persistent inflammation.
5. ↓ local tissue destruction and scar formation.

→ Block the production of all arachidonic acid derivates (eicosanoids) through induction the production of lipocortin.
→ >< arachidonic acid pathway → ↓leukotriene B4 (LTB4) → ↓peroxisome proliferator activated receptorα
(PPARα)→↓activity of activator protein (AP1) inflammatory cascade → ↓matrix metalloproteinase → ↓local soft
tissue destruction and scar formation.

Tretinoin

Topical tretinoin mechanism of actions were normalized differentiation and keratinization, prevented keratin plug formation. The action in the epidermis was restored of normal keratinization in conditions in which this was disurbed. It enhanced DNA synthesis in the germinal epithelium and increased the mitotic rate. It also had regulating effect on epidermal cell differentiation, leading to a thickening of granular layer and a normalization of keratinization in parakeratosis. Retinoic replaced of disorganized dermal collagen fibers, caused an increased in epidermal thickness and returned to a more uniform size and electron density of the basal and spinous keratinocytes. Retinoic decreased sebum production by reducing the size of sebaceous glands, in which the profoundly inhibited sebum excretion and affected comedo formation. Isotretinoin reduced 5α reductase activity in human liver and skin and reduced binding capacity of the androgen receptor in back skin [1,3].

Binding proteins for retinoids were widely distributed throughout the body in many cell types. Cellular retinoic acid binding protein II (CRABP II) predominated in the skin, and could be found in the keratinocytes and fibroblasts. Following transport to the nucleus, retinoid bound to retinoic acid receptor (RAR) and retinoic X receptor (RXR). Receptor interaction with DNA response elements stimulated promoter regions to induce transcriptional and ranslational activity. The resultant was protein synthesis, that in turn mediate structural and functional activity of the cells and modulate transcription of other genes [3].

Tretinoin was bound to nuclear receptor (RARs and RXRs) that acted as ligand activated transcription factor, Retinoid receptor complex modulated the transcription of spesific gene (DNA). Tretinoin receptor complex antagonized various transcription factors that was involved in proliferative and inflammatory responses such as AP1 (activator protein 1) or nuclear factor IL6 (NF-IL6) by competing for commonly required coactivator proteins, resulting in a non producing complex [1,3]. In arachidonic acid pathway, leukotriene B4 (LTB4) promoted skin inflammation by acting on peroxisome proliferator activated receptorα (PPARα). PPARα increased activity of activator protein (AP1) and NFkB led to recruitment of inflammatory cells. AP 1 inflammatory cascade lead activation of matrix metalloproteinase which contributed to local tissue destruction and scar formation [29].

Retinoic replaced of disorganized dermal collagen fibers, caused an increased in epidermal thickness and returned to a more uniform size

and electron density of the basal and spinous keratinocytes. For skin rejuvenation in photodamaged intrinsically and extrincally of the skin, at least 6 months of treatment is required. The predominance of one or other this effect depended on the concentration that be used. Erythema and irritancy in higher concentrations might be produced. It was still reported hypertrophic scars and keloids to respond to a daily application of a 0,05% solution [3].

Oral isotretinoids reduced sebum production by reducing the size of sebaceous glands, in which the profoundly inhibited sebum excretion and affected comedo formation. Isotretinoin reduced 5α reductase activity in human liver and skin and reduced binding capacity of the androgen receptor in back skin, but the mechanism of action was unknown. Isotretinoin reduced the synthesis of DNA and the corporation of a lipid precursor 14 C acetate in human sebaceous gland in vitro. After oral administration, it appear to influence the endogenous vitamin A in skin by increasing the concentration of retinol, but decreasing that of its local metabolite, 3-dehydroretinol, a substance was known to accumulate in hyperproliferating tissue. All trans retinoic acid had no this effect, because it was more rapidly metabolized than isotretinoin [3].

In acne topical tretinoin was normally applied at the concentration of 0,025-0,5% in gel, lotion or cream. Stronger preparation had been tolerated in dark skinned races. After an initial exacerbation, it caused softening and expulsion of comedos in 3-4 weeks, and would prevent from these reforming if it was continued. Neither erythema nor peeling were necessary for this to be achieved [3].

Topical all trans retinoic acid decreased epidermal melanin without melanocyte loss, acted as depigmenting agents [1,3].

Tretinoin was down regulated TLR2 & TLR4, decreased secretion of pro inflammatory mediators and increased T reg differentiation in innate and adaptive immune response in the later stages of acne inflammation processes cause decreased of persistent inflammation [1,3].

Tretinoin was down regulate FGFR2 that caused regulation cell cycles controled and influenced cellular progression, cellular differentiation, cell survival and apoptosis, decreased differentiation and hyperproliferation of keratinocytes and sebocytes, decreased lipogenesis, caused smaller the sebocytes size, decreased sebum production and alteration the composition of the cell surface lipid and attenuated growth and proliferation of infundibular leratinocytes [1,3].

Retinoids attenuated the FGFR2 pathway at several regulatory levels of the signal transduction cascade critical for cycle cell control, cell proliferation, differentiation and lipogenesis. Isotretinoin induced TGFβ (from T cell) caused terminating of keratinocytes and sebocytes activation cycle in acne vulgaris. Retinoid acid (9cis, 13 cis, all trans retinoic acid) increased TGFβ in dermal papillary caused inhibition of P53, increased of growth inhibition and increased hair follicle regression. Isotretinoin decreased FGFR2 signaling caused primary deactivation signal of activated keratinocytes and sebocytes in differentiation. Retinoids attenuated the FGFR2 pathway at several regulatory levels of signal transduction cascade, that caused decreased of cell cycle control, cell proliferation and differentiation and lipogenesis. Downstream FGFR2 signalling primary deactivated signal of activated sebocytes and keratinocytes [46].

Tretinoin was decrease TGFβ of Th 1 & T reg caused terminating keratinocytes & sebocytes activation cycles, decreased the expression of gen P53, increased growth inhibition, increased hair follicle regression [1,3].

Topical tretinoid unclogged pores by increased stickness of the keratinocytes inside follicle and decreased cell turnover, decreased sebum production, shrinked sebaceous glands and increased keratinocytes shedding [1,3].

Tretinoin blocked TLR2 activation directly, caused decreased primary cytokine IL1α (so decreased comedogenesis), cesreased IL1β & TNFα (so decreased persistent inflammation by stopping positive loop in NFKβ pathway). Tretinoin blocked TLR2 activation indirectly through NFkB pathway, caused decreased the production of secondary cytokine IL18 resulted decreasing of inflammation [1,3].

Isotretinoin and other retinoid down regulated TLR2 expression on monocytes. Furthermore , retinoids induced Treg differentiation and down regulated Th17 differentiation and neutrophyl migration, but is was unknown whether this mechanism were modulated by isotretinoin in acne. Isotretinoin also down regulated the expression of MMPs -9 and -13, which played a central role in inflammatory matrix remodelling. Finally retinoids stimulated the released of GM-CSF, monocyte chemotactic protein-1 (MCP-1) and IL10 in cultured monocytes and macrophages [10,54].

Retinoids attenuated the FGFR2 pathway at several regulatory levels of the signal transduction cascade critical for cycle cell control, cell proliferation, differentiation and lipogenesis. Isotretinoin induced TGFβ (from T cell) to activate Treg for secreting more TGFβ & IL10 that caused terminating of keratinocytes and sebocytes activation cycle in acne vulgaris. Retinoid acid (9cis, 13 cis, all trans retinoic acid) increased TGFβ in dermal papillary caused inhibition of P53, increased of growth inhibition and increased hair follicle regression. Isotretinoin decreased FGFR2 signaling caused primary deactivation signal of activated keratinocytes and sebocytes in differentiation. Retinoids attenuated the FGFR2 pathway at several regullatory levels of signal transduction cascade, that caused decreased of cell cycle control, cell proliferation and differentiation and lipogenesis. Downstream FGFR2 signalling primary deactivated signal of activated sebocytes and keratinocytes [46].

Steroid

Topical steroid acted as antiinflammatory, immunosupresive, anti mitogenic were due to their ability to exert multiple effects on the various function of the leucocytes, epidermal and dermal cells. Topical steroid decreased erythema (produced transient vasoconstriction) and irritacy that could be caused by topical tretinoin. Topical steroid avoided the possibility of hypertrophic scar and keloid that can be caused by daily application of 0,5% topical tretinoic [1,3].

Steroid upregulated TLR2 receptor, the activation TLR on macrophages by microbials ligands upregulated iNOS, which also result rapid generation of NO (caused decreasing of IL1β) and powerfull microbial activity.

Steroid mainly acted in central NFKB pathway to stop the production of primary cytokine positive loop activation that caused persistent inflammation (mainly caused by IL1β & TNFα) and secondary

cytokine (IL18) production. Steroid mainly acted in central NFKB pathway by increasing the gene expression of 1kBα thus serving to replace 1kBα degraded by 26Sproteosome after phosphorilation and ubiquitination and ultimately to sequester NFkB dimers in the cytoplasm as inactive complexes (NFkB-1kBβ tight bounding). Steroid tip the balance between sequestered and actived NFKB by inducing 1kbα synthesis. Primary cytokine (IL1β,TNFα) then promoted persistent inflammation by liberating NFkB from its association with 1kβ→increasing NFKβ released and nuclear translocation to nucleus for transcription→further increased secondary cytokine production (IL18), caused increasing of inflammation.1 In non stimulated cells NFKB heterodimers formed from p65 and p50 subunits are in active because they sequester in the cytoplasm as a result of tight binding to inhibitor proteins in the 1kB family. So NFkB did not release and there were no nuclear translocation for transcription, caused decreased cytokine production (IL1α,IL1β, TNFα) so decreased comedo formation and inflammation [1].

Glucocorticoid could lead to the inhibition of MHC class II antigen presentation pathways, which could downregulate immune responses leading to tissue injury and could also contribute to immunosupression [1].

The currently accepted general model of steroid action incorporates three major steps receptor binding, synthesis of spesific m RNA (transcription) and the synthesis of protein. Hydrophobic glucocorticoid mollecules diffuse across the plasma membrane of the cell and reversible bound to spesific receptor protein that was presented in the cytoplasm. This hormones (glucocorticoids) binding to the receptor caused increased DNA binding affinity due to allosteric changed in the structure of the receptor, leading to the accumulation of the steroid receptor complex in the cell nucleus. Gene transcription could be modulated by the hormonal complex binding to certain sequences on nuclear DNA termed hormone response element (HREs), which resulted the production of the new proteins by RNA mollecules using the new DNA complex as a template. Some of the new protein produced included lipocortin, Interleukine 1 and lymphokines (e.g. Interleukine2). Lipocortin a family of glycoproteins, plays an important role in regulating the activity of phospholipase A2, which subsequently effected the production and released of arachidonic acid, which was the precursor of leukotrienes and phospholipids [1,3].

Conclusion

Pro inflammatory mediators in acne had no protective role, it had harmfull effect caused persistent inflammation, soft tissue destruction and scar formation. Some drugs that acted as anti immunological reaction or anti pro inflammatory mediators were discussed as novel modality treatments for chronic acne inflammation processes.

Combination of tretinoin and steroid as topical therapy was rational and had a good result in acne vulgaris chronic inflammation processes. Topical tretinoin and steroid sated synergis, finally down regulated pro inflammatory production that caused decreasing inflammation, soft tissue destruction and scar formation.

Topical Tretinoin caused softening and expulsion of comedos in 3-4 weeks and would prevent from these reforming if its uses was continued, normalized differentiation and keratinization keratinocytes & sebocytes, prevented keratin plug formation, decreased cell turnover, increased keratinocytes shedding, decreased sebum production (shrinked sebaceous glands) & decreased comedo formation (by down regulated FGFR2 that caused down regulation of cell cycles control), replaced disorganized dermal collagen fibers that caused an increased in epidermal thickness, decreased epidermal melanin without melanocyte loss (acted as depigmenting agents), decreased persistent inflammation by down regulated of TLR2 & TLR4 that caused increasing of IL10 down regulated cytokine & TGFβ from Treg then caused terminating of keratinocytes & sebocytes activation cycles, decreased expression of gen P53 (anti keloids), increased growth inhibition,increased hair follicle regression [1,3]. Isotretinoin and other retinoid down regulated TLR2 expression on monocytes. Furthermore, retinoids induced Treg differentiation and down regulated Th17 differentiation and neutrophyl migration, but is was unknown whether this mechanism were modulated by isotretinoin in acne. Isotretinoin also down regulated the expression of MMPs -9 and -13, which played a central role in inflammatory matrix remodelling. Finally retinoids stimulated the released of GM-CSF, monocyte chemotactic protein-1 (MCP-1) and IL10 [10,54].

Topical Steroid acted as antiinflammation, immunosupression, anti mitogenic, decreased erythema (produced transient vasoconstriction), decreased irritacy that be caused by topical tretinoin. Glucocortikoid was upregulated TLR [10]. The activation TLR on macrophages by microbials ligands upregulated iNOS, which also result rapid generation of NO (caused decreasing of IL1β) and powerfull microbial activity. Macrophages used this mechanism to contain some infectious organisms that were not susceptible to PMN attacted such as mycobacteria, fungi, parasit and P acnes that had been resistant to phagocytosis. The stimulation of monocytes by activated T cells also led to generation of NO [1]. Steroid stopped central NFKB pathway and finally stop primary cytokine positive loop activation (IL1β ,TNF α) and secondary cytokine production (IL18) that caused persistent inflammation (IL1β ,TNF α) and comedo formation (IL1α). Steroid decreased local soft tissue destruction and scar formation by blocked the production of all arachidonic acid derivates (eicosanoids) through induction the production of lipocortin (chemokines). Steroids blocked arachidonic acid pathway, that caused decreased of leukotriene B4 (LTB4) that promoted skin inflammation by acting on peroxisome proliferator activated receptorα (PPARα) that caused increased activity of activator protein (AP1) inflammatory cascade and NFkB led to recruitment of inflammatory cells. AP1 inflammatory cascade led to activation of matrix metalloproteinase which contributed to local tissue destruction and and scar formation [1,3,29]. Topical steroid avoided the possibility of hypertrophic scar and keloid [3]. So steroid prevented from local tissue destruction and scar formation in acne vulgaris. Steroid acted as antiinflammatory, immunosupresive, anti mitogenic were due to their ability to exert multiple effects on the various function of the leucocytes, epidermal and dermal cells [3].

Topical steroid decreased erythema and irritancy that caused by tretinoin (erythema and irritancy). Topical tretinoin supressed topical steroid side effects, such as acneiform eruption, acne steroid, epidermal thinning, reducing collagen synthesis.

Single topical tretinoin as maintenance treatment was good. The effect of long term maintenance treatment with topical tretinoin was decreasing sebum production, decreasing hypercornification & epidermal turnover, increasing keratinocytes shedding, decreasing

the expression of gen P53 (anti keloids), increasing of collagen formation (good for skin rejuvenation), inhibiting melanin transferred (good for skin brighter).

Brief Conclusion

The combination of tretinoin and steroid as topical anti immunological reaction (anti pro inflammatory mediators) therapy for acne chronic inflammation processes were rational and had good result. They acted synergis, finally down regulated cytokine production, decreased inflammation, soft tissue destruction and scar formation. Single topical tretinoin as long term maintenance treatment was good

The local side effect of tretinoin such as erythema and irritancy could be prevented by steroid, and steroid side effect such as acneiform eruption, acne steroid, epidermal thinning, reducing collagen synthesis could be prevented by tretinoin.

Steroid (acted in NFkB pathway and upregulated TLR2 receptor) and tretinoin (antagonist TLR2 receptor and FGFR2) were easy to get, cheap and safe in combination for short term periods (4 weeks). Tretinoin decreased comedo formation, sebum production & hyperkeratinization, normalized keratinization and differentiation, replaced disorganized collagen fibers & formed thicker-elastic epidermis (for skin rejuvenating), diminishing PIH- inhibiting melanin trasferred (for skin brightening), decreasing the expression of gen P53 (anti keloids), decreased scarring (directly down regulated the expression of MMP). Steroid decreased chronic persistent inflammation, prevented from hypertrophic scar and keloid formation, decreased erythema & irritancy.

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