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REVIEW ARTICLE

Papulopustular rosacea, skin immunity and Demodex: pityriasis folliculorum as a missing link

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Abstract

Papulopustular rosacea (PPR) is a common facial skin disease, characterized by erythema, telangiectasia, papules and pustules. Its physiopathology is still being discussed, but recently several molecular features of its inflammatory process have been identified: an overproduction of Toll-Like receptors 2, of a serine protease, and of abnormal forms of cathelicidin.

The two factors which stimulate the Toll-like receptors to induce cathelicidin expression are skin infection and cutaneous barrier disruption: these two conditions are, at least theoretically, fulfilled by Demodex, which is present in high density in PPR and creates epithelial breaches by eating cells. So, the major pathogenic mechanisms of Demodex and its role in PPR are reviewed here in the context of these recent discoveries.

In this review, the inflammatory process of PPR appears to be a consequence of the proliferation of Demodex, and strongly supports the hypothesis that: (1) in the first stage a specific (innate or acquired) immune defect against Demodex allows the proliferation of the mite; (2) in the second stage, probably when some mites penetrate into the dermis, the immune system is suddenly stimulated and gives rise to an exaggerated immune response against the Demodex, resulting in the papules and the pustules of the rosacea.

In this context, it would be very interesting to study the immune molecular features of this first stage, named ''pityriasis folliculorum'', where the Demodex proliferate profusely with no, or a low immune reaction from the host: this entity appears to be a missing link in the understanding of rosacea.

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Introduction

Papulopustular rosacea (PPR) is a common facial skin disease, characterized by vascular (flushes, erythema and telangiectasia) and inflammatory (papules and pustules) symptoms. Its physiopathology is multifactorial and is still being discussed.

Several molecular features of its inflammatory process have been recently identified: in 2007, Yamasaki *et al.*[1] observed that individuals with rosacea show an increase in both serine protease kallikrein (KLK5) and abnormal forms of cathelicidin in the facial skin. Moreover, they showed that two of these cathelicidin peptides (LL-37 and FA-29) induce erythema and vascular dilation when injected in the skin of a mouse.[1] LL-37 has a lower antimicrobial power than the smaller cathelicidin found in healthy skin,[2] and, especially, it promotes more angiogenesis and inflammation, leading to the clinical findings of rosacea.[1] In 2011, Yamasaki *et al.*[3] showed that Toll-like receptors(TLR) 2 expression is also increased in rosacea, and that it stimulates enhanced KLK5 production in a calcium-dependent manner. They suggest that the origin of PPR is a disorder of the immune system: for them, the initial increase of TLR2 explains why rosacea patients might overreact, although bacterial diversity and quantities are similar between rosacea and normal skin.[3]

As already suggested by Bevin and Liu[4] concerning LL-37 and KLK5 in 2007, this hypothesis does not explain the initial increase in TLR2 and, in particular, whether this occurs as a response to specific triggers. Although the bacteria are quite similar in rosacea to normal skin, this is not the case with the Demodex (D) population. However, the two factors that stimulate the TLR to induce cathelicidin expression are skin infection and cutaneous barrier

disruption:[5] these two conditions are, at least theoretically, fulfilled by D, when it proliferates and eats epithelial cells. Moreover, the increase in TLR2 could be explained by a secondary positive feedback, as the vitamin D-dependent amplification mechanism, described by Schauber *et al.*[5]

Since 1925,[6] the role of D in PPR has given rise to debate: at present, D is usually considered as playing a pathogenic role when it multiplies[7–25] and when it penetrates into the dermis.[26–34] However, many dermatologists do not agree with this concept when the clinical condition is the PPR.

For several reasons, it is impossible to establish the pathogenicity of D using the Koch postulates: (i) it parasitizes the healthy skin; (ii) its pathogenicity depends on the immunological ground of the patient; (iii) as it is an obligate parasite, it cannot be grown *in vitro* and so a massive experimental infestation can therefore not be performed.[35] However, not all accepted pathogens fulfil these postulates;[36] so, in this article, we shall review the literature to see if we can establish the pathogenic role of D in PPR, without fulfilling the Koch postulates.

Demodex and its major pathogenic mechanisms

Demodex

Demodex is a spindle-shaped transparent mite (Fig. 1b) which lives exclusively in the pilosebaceous follicles of mammals.[37] Its role remains unclear: in particular, we do not know whether it confers some benefit to the host, or not.[38] Each species of D is specific to its host species.[35] In the human skin, two species of D are observed: *D. folliculorum*, a long form which lives in the pilosebaceous duct, and *D. brevis*, a short form which inhabits the sebaceous and Meibomian glands. Over their life-cycle of ±15 days, male and female change from ovum to larva, to protonymph, to deutonymph and then into adult form,[39] varying in length between 0.06 and 0.4 mm.[37] Copulation occurs in the mouth of the follicle.[39] *D. folliculorum* is observed in normal skin with a prevalence of 100%[7,8] and a density of ≤5 D/cm^2 in the adult population.[7]

As a result of its deep location, *D. brevis* is not easily detectable by a non-bleeding sampling method, and is therefore less well known than *D. folliculorum*. This is the reason why, in this review, we principally consider *D. folliculorum* even if *D. brevis* probably plays a similar pathogenic role in PPR.

Demodex causes a cutaneous barrier disruption

The mite, moving with four pairs of legs, each of them bearing two claws,[40] is responsible for the erosion of the epithelium.[41] In eating the human cells,[40] D is responsible for even more important cutaneous breaches[30] (Fig. 2a). Even, when eating cells transversally,[30] it can penetrate into the dermis[24,26,28,30] (Fig. 2b): as the cutaneous barrier becomes disrupted, the TLR are, as a rule, stimulated[5] and the D antigens are exposed to the human immune system. Further studies are needed to confirm that D really does stimulate TLR.

So, D is responsible for a permanent micro abrasion of the skin, which is likely to be the origin of the hypersensitivity encountered in rosacea. This observation is corroborated by our clinical experience: the complaint of sensitive skin disappears concomitantly with the normalization of the D density (Dd).

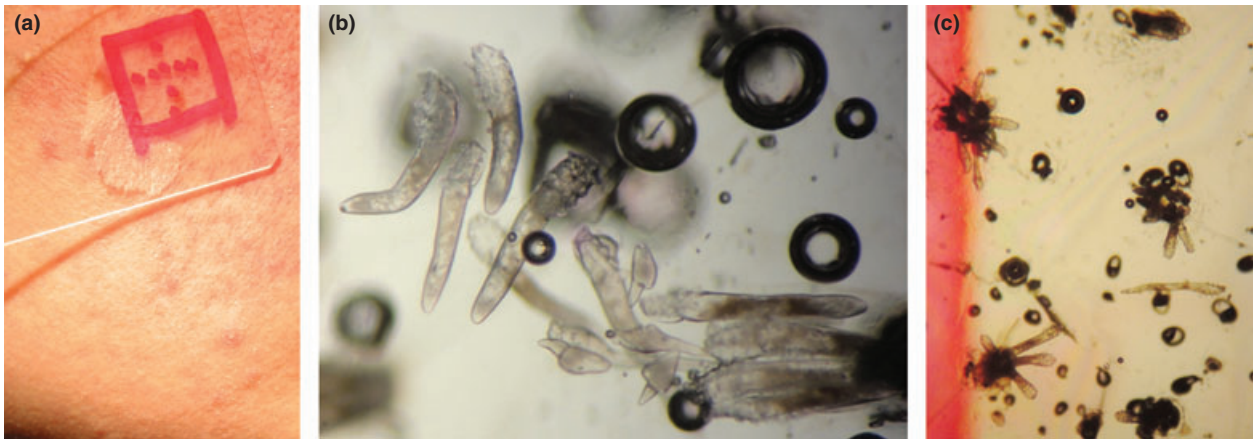

Figure 1 (a) The Standardized Skin Surface Biopsy[7,64,65] is a non invasive sampling method by which it is possible to collect 1 cm^2 of the superficial part of the horny layer and of the follicle content. It consists of placing a drop of cyanoacrylic adhesive (Loctite®) on a microscope slide, applying the adhesive bearing surface of the slide to the skin, and removing it gently after it has been allowed to dry (about 1–3 min). Initially, a standard surface area of 1 cm^2 is drawn on the slide with a red waterproof pen, traced by transparency after putting down the lamina on a sheet of standard square paper. The area of 1 cm^2 is also divided into four equal squares to make the further counting of parasites easier. After removal from the skin, each sample is clarified with 2–3 drops of immersion oil, and then covered with a cover slip. The samples are studied microscopically at standard magnifications (×40, ×100). (b, c) Direct microscopic examination during the consultation: (b) Demodex folliculorum (±12 adults, 2 larvae, 3 eggs) free and inside the follicle (×100). (c) Five follicles with 3–9 Demodex folliculorum per follicle (×40).

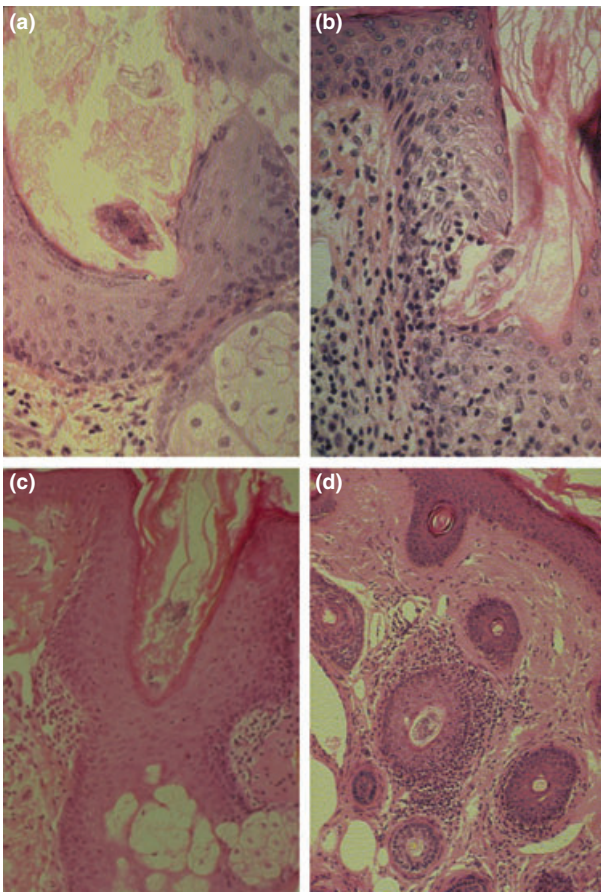

Figure 2 Cutaneous biopsies (hemalun-eosine-saffron). (a) Demodex is cut slantways in a pilosebaceous duct. Just in front of the mite, the granular layer of the duct is interrupted because the D is eating the human cells (×475). (b) Demodex folliculorum is cut longitudinally in the follicular duct. Eating the human cells transversally, it penetrates more deeply through the epithelium. So, we can see its trajectory: the mite is actively passing through the follicular wall, to penetrate into the dermis (×475). (c) Demodex folliculorum is cut longitudinally in the follicular duct. The perifollicular infiltrate is often localized at the level of the gnathosome of the mite, with which it causes the most significant epithelial breach (×200). (d) Follicles are cut transversally: the follicle which is the most attacked by a lymphocytic infiltrate contains a Demodex. This is not a coincidence, it illustrates the reality: the lymphocytic infiltrate is actually statistically related to the presence of Demodex inside the follicle[30,46] (×475). Figures 2a, 2b, and 2c are reprinted from ''Forton F. Demodex et inflammation périfolliculaire chez l'homme : revue et observation de 69 biopsies. Ann Dermatol Vénéréol 1986; 113: 1047-58.3'' 30, with the authorisation of Elsevier Masson S.A.S., Paris.

Preoral digestion:[40] release of enzymes (protease?)

To eat, D projects two stylets from the preoral opening to puncture the host cells[35,40] (Fig. 3): *D. folliculorum* has special piercing mouthparts, while *D. brevis* has a simpler structure.[42] It scrapes the internal surface of the host cells with two palps,[40] and secretes enzymes from two salivary glands for a preoral digestion: the liquefied cytoplasm is then ingested into the food canal by the action of the pharyngeal pump.[40] It may be supposed that there are proteases amongst its salivary enzymes. This hypothesis is supported by the observation, in cutaneous biopsies, of two anti-proteases on the cuticle of *D. folliculorum* and *D. brevis*, as if they were a protective response by the human host against the mite infestation.[43] Indeed, in atopic dermatitis, two proteases derived from house dust mites are identified as causing skin irritation or immune activation.[44] The D proteases might interfere with the endogenous protease/protease-inhibitor balance and lead to exacerbations of PPR, probably like the house dust mite proteases do in atopic dermatitis.[45]

Demodex induces a type IV immunological reaction

In cutaneous biopsies, the presence of D inside the follicles is statistically correlated with a lymphocytic infiltrate around these follicles[30,46] (Fig. 2c,d). When D passes through the follicular wall and penetrates into the dermis, a granulomatous reaction occurs and giant cells phagocytize the mite, which is observed in the granulomatous rosacea.[24,26,28,29,47] It is interesting to note here that the granuloma are observed in biopsies not only in clinical granulomatous rosacea, but also in PPR,[32,47] and even in erythematotelangiectatic rosacea.[47]

Authors, in general, still consider that D passes into the dermis passively, after the destruction of an infested follicle by the inflammation.[26,47,48] However, the reason why the inflammatory process destroys the follicle is precisely the presence of numerous D inside the follicle.[26,47] The penetration of the mite into the dermis does not occur at random: D penetrates the dermis actively (Fig. 2b) or passively, thanks to the inflammation that it itself induces.

On the other hand, it has been demonstrated that the lymphocytes of the infiltrate around the infested follicles of PPR and those of D granulomas are both T helper cells.[49,50] Probably, they are T helper-17 cells, known to produce interleukin-17 and to activate the TLR.[51]

Moreover, some isolated cases of D proliferation are also reported amongst patients with cellular immunodeficiency of various origins. However, the most interesting fact is that, in several patients, the papules and pustules appear when immunity begins to be restored by the treatment.[52]

All these studies suggest a type IV immunological reaction.[30,49,50]

For some authors, the perivascular location of the infiltrate (also observed in PPR)[47] suggests that rosacea is not a follicular but a vascular process. However, in addition to the perifollicular infiltrate around the infested follicles, the perivascular location of the infiltrate can be explained very well by the first stage of a type IV immunological reaction against D: T helper lymphocytes and macrophages are attracted by antigens and appear around the vessels of the infected site.

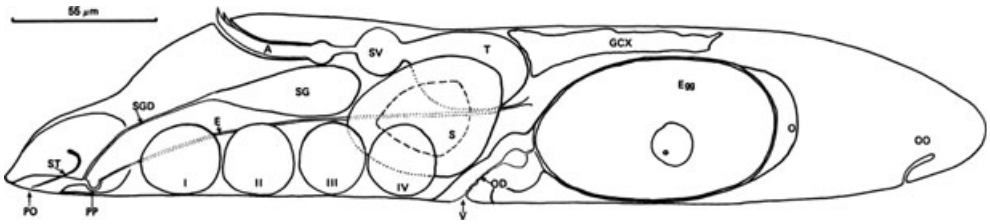

Figure 3 Diagrammatic representation of the anatomy of Demodex spp. showing: A = penis, E = oesophagus, egg, OD = oviduct, GCX = gut cells with crystals, O = ovary, OO = opisthosomal organ (function unknown), PO = preoral opening, PP = pharyngeal pump, S = brain, SG = salivary gland, SGD = salivary gland duct, ST = stylets, SV = seminal vesicle, T = testis, and V = vulva.[35] Roman numerals designate leg bases (diagram based on Desch and Nutting,[40] 1977).[35] This figure is reprinted from ''Nutting WMB, Andrews, JRH, Desch CE. Studies in symbiosis: hair follicle mites of mammals and man. Journal of Biological Education 1979; 13: 315–21.'' copyright © The Society of Biology, with the permission of (Taylor & Francis Ltd, http://www.tandf.co.uk/journals) on behalf of The Society of Biology.

Relation between D and bacteria, and skin temperature

Papulopustular rosacea pustules are known to be sterile: the bacteriological samplings (direct examination and culture) show only commensal flora.[53] Whitfeld *et al.*,[54] analysing 15 PPR, observed that the unique pure growth culture found was *Staphylococcus epidermidis*, and found this positive culture in nine patients, but only in their pustules, and on the inferior eyelid of four patients (not in the control subjects). As suggested by Yamasaki *et al.*,[1] this proliferation could be induced by the immune profile observed in PPR (LL-37 with low antibacterial power[2]).

Skin temperature could explain why commensal microorganisms become pathogens in PPR, as it influences the behaviour of bacteria[55] and Demodex.[56] *Staphylococcus Epidermidis* isolated from patients with rosacea secretes more proteins (of which a lipase) at 37° compared with 30°.[55] The motility of D increases with temperature: at 37°C, the mites are extraordinarily active, but have a shorter survival time.[56] Perhaps an optimal temperature favouring D copulation exists? But this attractive hypothesis, relating the vascular and inflammatory processes of the disease, has not yet been confirmed: indeed, even if skin temperature does increase during flushes,[57] no significant difference in skin temperature is observed between either ETR or PPR affected areas and healthy controls, and between affected and non-affected areas both in the ETR as in the PPR group.[58]

Some authors have investigated the hypothesis of bacteria carried inside the Demodex:[59,60] Borgo *et al.*[60] tried to detect the bacterium *Wolbachia* within human and canine D mites, using PCR analysis: in none of the DNA extracts, was the bacterium detected. Lacey *et al.*[59] studied the role of the *Bacillus oleronius*, potentially ingested by D, in PPR: they analysed 40 D [1 D extracted from each patient with PPR (n = 40)]. They found only one specimen of this bacterium in only one of the 40 D. However, they showed that two Ag of this bacillus activated the peripheral blood mononuclear cell proliferation in 16/22 patients (73%) with rosacea and in 5/17 controls (29%) (P = 0.0105).[59] They suggested a prior sensitization to this bacterium in PPR patients.[59] Another study supports this hypothesis by finding a significant statistical correlation between serum immunoreactivity against this bacterium and rosacea.[61] Owing to their increased TLR, rosacea patients might respond more than normal to commensal bacteria of the skin, and this could explain why different microbes could contribute to the disease.[3]

All this suggests that the proliferation of D induces an exaggerated immune reaction which indirectly (i) favours the proliferation of *Staphylococcus epidermidis*, and (ii) enhances an immune reaction against different types of microbes (as *Bacillus oleronius*).

On the other hand, bacteria could also influence D proliferation. Indeed, in 2009, Lai *et al.*[62] showed that *Staphylococcus Epidermidis* is able to modulate the inflammation, by the secretion of lipoteichoic acid; this molecule has distinct effects depending on the cells type exposed: an anti-inflammatory action on keratinocytes, but a proinflammatory action on immune cells that normally exist in a sterile environment. Through this mechanism, the commensal bacteria are tolerated on the epidermal surface without initiating inflammation.[62] As this molecule inhibits inflammation triggered by injury through a TLR2 dependant mechanism,[62] it could, at least theoretically, also increase the tolerance for D. On the other hand, D, by its abrasive action, could favour the contact of this molecule with the dermis, and so, its proinflammatory action. This could be an explanation as to why D proliferate at a first stage, and produce an exaggerated immune reaction at a second stage.

Demodex and rosacea

Infection: high Demodex density in papulopustular rosacea

Since 1993, it has been demonstrated that the Dd is higher on the cheeks of patients with PPR than on the cheeks of control patients with healthy skin.[7–11,63] Using the Standardized Skin Surface Biopsy (SSSB)(Fig. 1), a mean of 12.8 D/cm^2 is observed on the cheeks of patients with PPR, vs. 0.7 D/cm^2 for the control patients

($P < 0.001$).[7] If the skin surface and the slide are cleaned with ether before performing the SSSB, an even higher Dd is obtained in patients with PPR (m = 36 D/cm^2).[64] Moreover, PPR with normal Dd are rare and are probably induced by false negative results.[64,65] Most of the D observed by SSSB are *D. folliculorum*, because *D. brevis*, which mainly lives deeper in the sebaceous glands, is rarely observed with this sampling method. We can suppose that *D. brevis* also proliferates in the sebaceous and Meibomian glands, being responsible for deeper papulo pustules, especially on the nose, and of chalazion[25] of the ocular rosacea, but this has not yet been proved.

Similarity between PPR and canine demodicosis

In veterinary medicine, the pathogenic role of D is not disputed, especially in dogs in which *D. canis* (and two other species of D recently recognized in some dogs) is responsible for a well-known and potentially fatal disease.[66] Whether a dog develops demodicosis depends on immunological factors that are affected by genetic influences: the great majority of clinical cases are seen in purebred dogs that are otherwise in generally good condition. A hereditary *D. canis*-specific T cell defect, of varying severity, is incriminated.[66–68]

The prognosis clearly depends on the cellular immunity. If a cell mediate immune reaction[67] occurs, the disease spontaneously clears up: this is Localized Canine Demodicosis.

On the contrary, if there is no immune reaction, the proliferation of D is not curbed; moreover, as do most parasites, *D. canis* increases immunosuppression, creating a vicious circle.[66,67] Later, the mites are released into the dermis where a granulomatous reaction occurs, with giant cells phagocytizing the mite. From the dermis, dead D are carried in the blood to internal organs. This condition is often complicated by a bacterial septicaemia.[66] This is Generalized Canine Demodicosis.

A lot of similarities can be observed between PPR and Generalized Canine Demodicosis: (i) like in the dog, more than one species of D are observed in the human skin; (ii) like in the dog, the great majority of PPR occurs among patients who are otherwise immunocompetent;[64] (iii) like in the dog, the pathogenicity of *D. folliculorum* is related to its proliferation and to the immune response of the host; (iv) like in the dog, interactions probably exist between D and bacteria; (v) both from a clinical and a histological point of view,[66,69] PPR and the Generalized Canine Demodicosis look very much alike (Fig. 4).

Acaricidal creams treat PPR

Various authors report observing rosacea with a large number of D; most of them observe a decrease in the number of D following acaricidal treatments, concurrent with clinical improvement.[13,16,19,70,71] This is what we have observed in our daily clinical practice for more than 10 years (Fig. 5). These are of course observational studies, they should be confirmed by clinical trials (on PPR using acaricidal treatments on D).

Nevertheless, the PPR localized on the nose usually show an incomplete response to the acaricidal creams and often require oral isotretinoin. This kind of rosacea is probably more closely linked to *D. brevis* proliferation: their deeper location in hypertrophic sebaceous glands of the nose could explain their stronger resistance to the topical acaricidal treatment, which is applied on the skin surface.

Pityriasis folliculorum: first step to PPR

The clinical entity 'pityriasis folliculorum' was described by Ayres in 1930. It consists of very small, discrete and regularly dispersed follicular scales, which often induce a false sensation of dry skin[12,15] (Fig. 6a). In practice, patients come to the dermatologist with mainly subjective complaints (such as a sensation of pruritus, dry

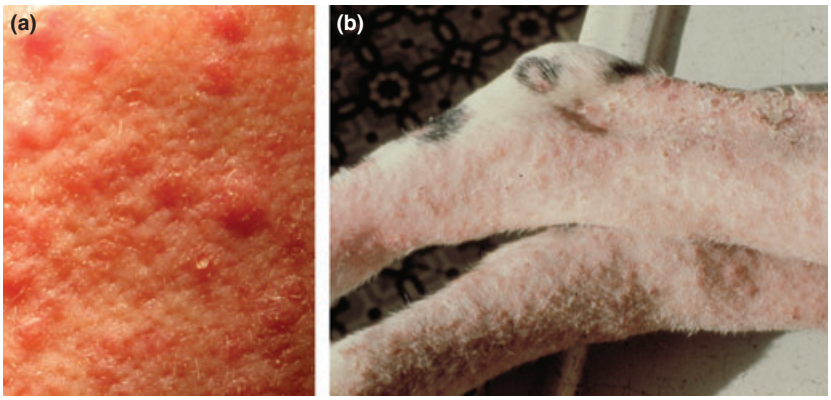

Figure 4 (a) Cheek of a woman with papulopustular rosacea. (b) Legs of a dog with Generalized Canine Demodicosis. From a clinical point of view, PPR and the Generalized Canine Demodicosis look very much alike: the deep violet papules of the Generalized Canine Demodicosis giving a mamillated aspect to the skin of the dog, closely resemble the papules of the patients with rosacea. The Figure 4b was given by Professor M Henroteaux, faculty of veterinary medicine, University of Liège, and is published with his permission.

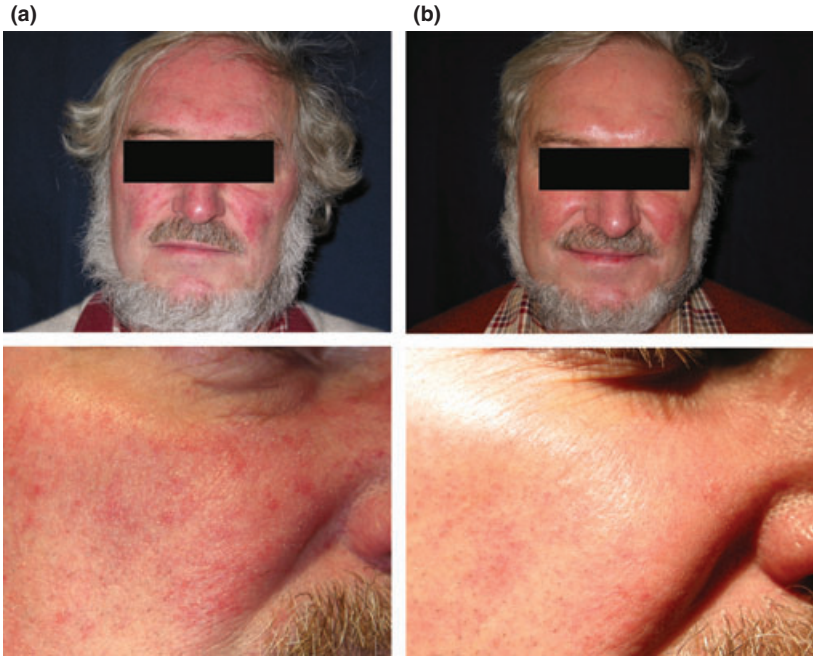

Figure 5 (a) 64 year old male patient presenting a papulopustular rosacea, present for the last 20 years. Before treatment, two successive standardized skin surface biopsies in the same place, on the cheek, reveal, respectively, 78 D/cm^2 and 204 D/cm^2. (b) After 2 months of local acaricidal treatment only, the skin is healthy and the two successive standardized skin surface biopsies in the same place on the cheek reveal, respectively, 0 and 0 D/cm^2.

skin, hypersensitive skin, irregular or rough skin) and the dermatologist is able to observe this very discrete picture only on close examination. Sometimes, the skin can even have a greasy aspect because the thin dry scales are covered by a layer of sebum. The diagnosis is made easier by cleaning the skin with ether and by tangential illumination: the thin whitish follicular scales then become more visible. They can be localized on the face, but also on the eyelids, the ears, the neck and the scalp: the equivalent of the facial follicular scales on the eyelids are cylindrical scales around the base of the lashes, [23,72] and, on the scalp, they are a kind of dandruff.

The usual vascular symptoms described (diffuse flushing or erythema with follicular accentuation)[12,15,73] have been observed in only 77% of our patients with pityriasis folliculorum:[64] although frequently associated, vascular symptoms are facultative to retain this diagnosis.

We have observed that this condition is more frequent than PPR: it is the most frequent demodicosis (54%),[64] but, as it is discrete, and not well known, it is usually[24] not properly diagnosed. In pityriasis folliculorum, which is characterized by discrete inflammatory symptoms (erythema), we observe a very high Dd (61 D/cm^2, $n = 45$) compared with a relatively lower Dd in PPR (36 D/cm^2, $n = 31$), which is characterized on the contrary by an important inflammatory response (papules, pustules) ($P = 0.04$)[64] (Fig. 6). Patients with pityriasis folliculorum are also a little older than the others[64] and it is known that older patients have a lower immunity. All this suggests that, in pityriasis folliculorum, a low immune reaction is responsible for a larger proliferation of D, and in PPR, the more intense immune reaction impairs the D proliferation, but without being completely effective, because it does not succeed in eliminating the mites.

Moreover, all intermediate degrees exist between pityriasis folliculorum and PPR: only discrete pityriasis folliculorum, the apparition of some papules and pustules, and true PPR (Fig. 6): papules appear on a ground of pityriasis folliculorum.

So, we could compare the pityriasis folliculorum with the first stage of the Generalized Canine Demodicosis,[67] where cellular reaction fails until hair follicle rupture.

Discussion

Direction of the causal relationship: Demodex-PPR

We analyse the direction of the causal relationship at different levels (molecular, histologic, clinic): does the inflammatory reaction of the PPR induce the D proliferation? Or does the D proliferation induce the inflammatory reaction?

1 Yamasaki *et al.*[1,3] suggest that rosacea is originally a disease of the immune system: the immunological changes observed (LL-37), occurring at a first stage, could therefore favour D proliferation at a second stage. In this case, we should observe: (i) a higher Dd when inflammatory symptoms are intense (because LL-37 is responsible for the inflammatory symptoms); (ii) a large number of patients with PPR without high Dd, (iii) some of whom develop a pityriasis folliculorum secondarily. In reality, it's the contrary: (i) the highest Dd occurs when the clinical symptoms are the lowest; (ii) nearly all of our PPR (88% (42/48)) have high Dd;[64] (iii) and we observe a large number of patients with pityriasis folliculorum (more frequent than PPR),[64] some of whom only secondarily develop a PPR.
2 Could the high Dd be an epiphenomenon, secondary to the perifollicular infiltrate? Considering the usual host-

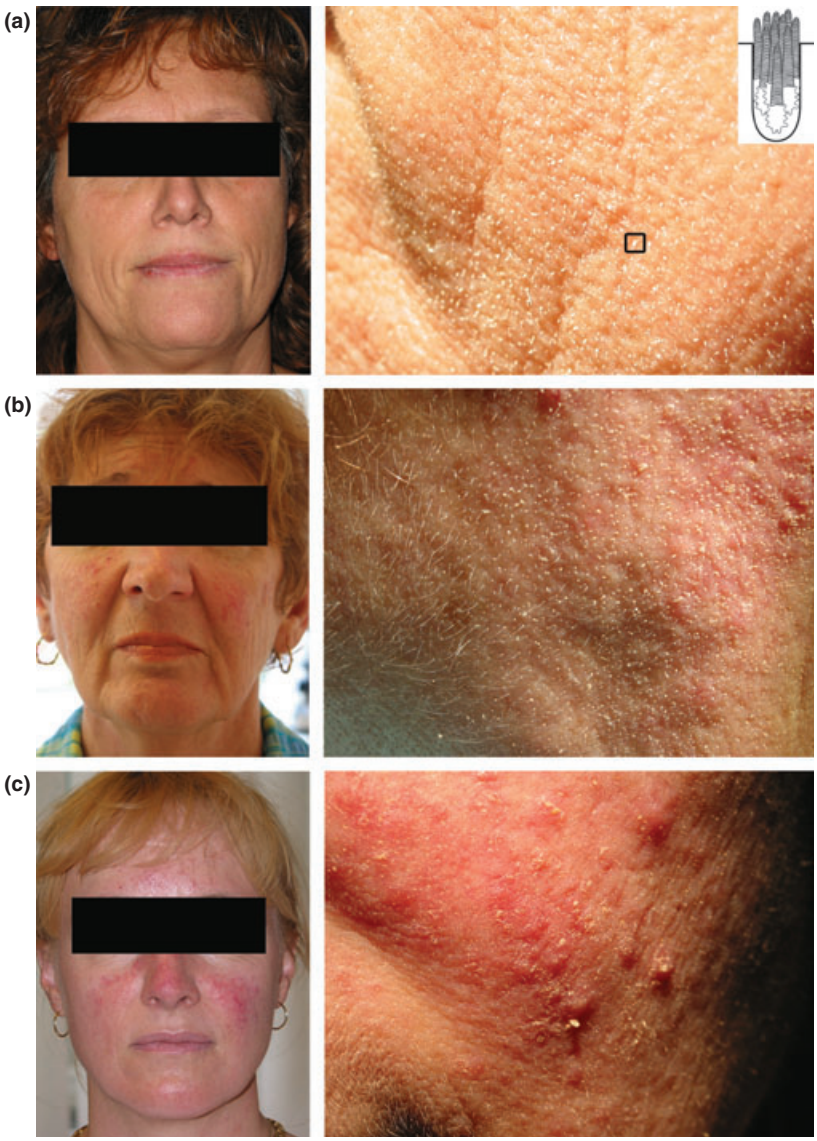

Figure 6 Patients with different levels of inflammation, with the values of two successive standardized skin surface biopsies performed in the same place on the cheek. The more intense the inflammatory reaction, the lower the Demodex density.[64] (a) Pityriasis folliculorum on a 50 year old woman with, respectively, 143 and 640 D/cm^2. Typical relatively discrete fine, dry and whitish scales, spread regularly over the face, with typical frosted appearance and rough texture.[12,15] Each of these fine follicular scales (square) is nothing other than a group of opithosomes of Demodex protruding externally (insert). In spite of a high Demodex density, no clinical inflammatory reaction is observed. (b) Discrete papulopustular rosacea: telangiectasia, hillocky skin, and a few papules appear on the pityriasis folliculorum of a 71 year old woman, with 124 and 188 D/cm^2. (c) Papulopustular rosacea: erythema, follicular and non-follicular scales, deep and large papules, on a 48 year old woman, with two and 20 D/cm^2. The inflammatory symptoms are important and the Demodex density, although high, is relatively moderate compared with the other cases.

microorganism relations in medicine, the probability that D induces the perifollicular inflammation is higher than the converse: why should the mites proliferate in inflamed follicles?

3 Does the breach in the epithelium create the presence of the mite or does the mite create the breach? When we see the anatomy of D (Fig. 3), and the biopsies (Fig. 2a,b), it is obvious that D creates the breach, and might even actively pass through the follicular wall, and penetrate into the dermis.

4 Moreover, if the mite proliferation is a consequence of clinical lesions, D should proliferate in other close hyperkeratotic and inflamed facial skin diseases (as lupus erythematous and ulerythema ophryogenes), which is not the case: so, the erythematosquamous condition is not the cause of the proliferation of the mite.

5 Finally, if D is not the cause of the inflammation, how could we explain clinical cures of rosacea by acaricidal treatments, concomitant with a decrease in the number of D?

All these arguments, (1) the apparent inverse proportion between LL-37 and Dd, (2) the perifollicular and granulomatous reaction which is a consequence of D, (3) the breach created by the mite, (4) the absence of D proliferation in other diseases, and (5) the action of acaricidal treatments on clinical lesions, strongly support the thesis that PPR is indeed caused by D and not the opposite.

Nosologic question

Several dermatologists recognize the role of D in Demodicosis Rosacea-Like, but not in PPR: they want to distinguish PPR from

demodicosis. This partition does not withstand the analysis. First, the clinical differences are very relative: (i) the pustules are described as being bigger in PPR.[15] In practice, PPR only with deep pustules is rare: most of the time pustules of differing size are observed together, the size depending on the depth of the infiltrate (bigger when deeper). The deepest pustules could be related to the proliferation of *D. brevis* in the sebaceous glands. (ii) The follicular scales of the Demodicosis Rosacea-Like[15] are almost always observed in case of PPR too, if the dermatologist searches for it by close examination after cleaning the skin with ether.

Secondly, in daily practice, PPR without high Dd are very rare:[64] if PPR without D proliferation existed, it would be a rare entity, almost never observed by dermatologists. However, the diagnosis of rosacea is frequently made. This means that the PPR diagnosed today are, in reality, demodicoses.

But why does Demodex proliferate in certain patients and not in others?

Akilov and Mumcuoglu[74] have published several immunological studies about the statistical relationship between demodicoses and immunological characteristics: they have studied HLA haplotypes, peripheral blood circulating lymphocytes,[75] and have highlighted an immunosuppression amongst the patients with demodicoses. They have observed an association between the frequency of HLA Cw2 and Cw4 haplotypes and human demodicosis,[74] and have demonstrated that an increasing Dd is associated with an increasing trend of apoptosis in lymphocytes.[75] This immunosuppression could be a genetical predisposition facilitating the survival of the D, but could also be a secondary local immunosuppression, induced by the mite itself.[75]

On the other hand, we observe that demodicoses are frequent in a healthy population, occuring among immunocompetent patients.[64] The particular immunological ground, favouring D proliferation, is certainly frequent in a healthy population, and is therefore to be considered as a mite-specific deficiency, and not as a deep immunosuppression, as in the canine demodicosis.

This genetically determined immunological ground could explain the relatively high frequency of the familial cases of rosacea and other demodicoses.

Not only innate immunosuppression but also acquired immunosuppression seem to favour D proliferation: Seyhan *et al.*[76] have observed a higher incidence of high Dd (≥5 D/cm^2) in patients receiving chemotherapy than in a control group, and Karincaoglu *et al.*[77] have observed that the Dd is higher in patients with end

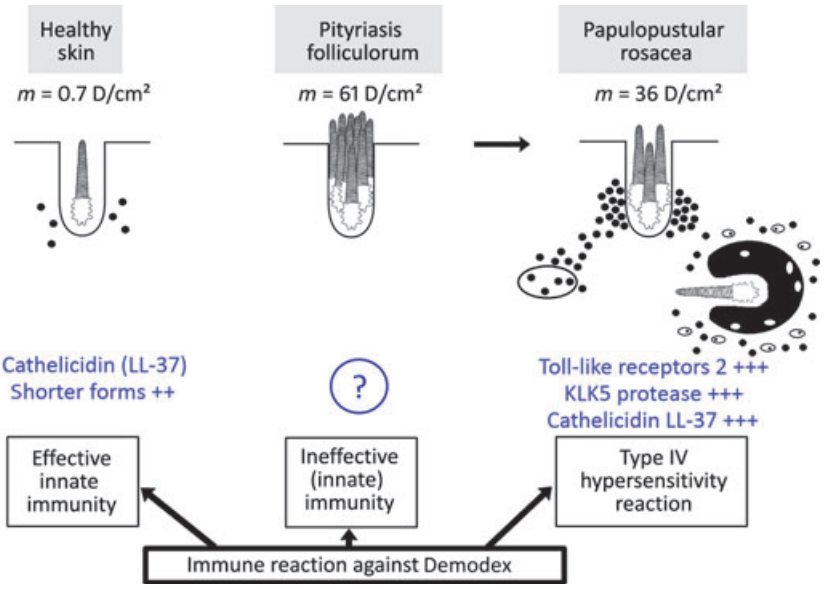

Figure 7 Physiopathology of papulopustular rosacea. Schematic representation assembling data from the recent molecular discoveries[1,3] with the data concerning the Demodex in rosacea.[7–13,15,16,19,24,26,28–30,47,49,50,63,64,74,75] (i) In healthy skin, the immunity is normal, with normal cathelicidin (rare LL-37; shorter forms predominating):[2] the immunity controls Demodex proliferation. (ii) In pityriasis folliculorum, in spite of a very high Demodex density, no significant inflammatory clinical reaction is observed: the cell-mediated immune response is absent or mild, probably due to a genetically determined specific defect of the innate immunity,[74] and aggravated by an immunosuppression induced by the Demodex.[75] (iii) In papulopustular rosacea, the immune reaction is exaggerated with increased Toll-Like receptors,[3] excessive production of KLK5 and cathelicidin LL-37,[1] and a type IV hypersensitivity reaction,[30,49,50] which is responsible for the formation of the papules and pustules clinically. This immune reaction partially limits the proliferation of the mite, without being completely effective. If the Demodex penetrates into the dermis, a granulomatous reaction occurs.[24,26,28,29,47] In daily practice, the third condition appears on a ground of pityriasis folliculorum.

stage chronic renal failure (6.12 D/cm^2, $n = 62$) than in the control patients (0.31 D/cm^2, $n = 62$) ($P = 0{,}000$). Other factors like erythematotelangiectatic rosacea, skin temperature,[56] lack of soap,[12,15,64,70] or thick layers of make-up,[15,70] and sebaceous hyperplasia could also favour D proliferation.

Conclusion: pityriasis folliculorum as a missing link

All these studies and arguments confirm the pathogenic role of D in the inflammatory lesions of PPR, and support the hypothesis that: (i) at a first stage, a specific (innate or acquired) immune defect against D allows the proliferation of the mite (which probably itself increases the local immunosuppression); and (ii) at a second stage, after months or years, probably when the breaches in the epithelium become significant, or, as in dogs, when some mites penetrate into the dermis, the immunity is suddenly stimulated and produces an exaggerated (and not very effective) immune response, inducing the apparition of the papules and the pustules of PPR (Fig 7).

In this context, three different approaches appear to treat PPR. They are in order of increasing relevance, but can be complementary: (i) to struggle against the exaggerate immune reaction [according to the current guidelines (with tetracyclines and metronidazole) and also as proposed by Yamasaki *et al.*[1] (by influencing the balance of antimicrobial peptides)], (ii) to kill the mites (which is what we do with local acaricidal treatments), and (iii) to help patients themselves limit D proliferation (this is the way of the future).

To this purpose, it would be very interesting to identify the immune defect which allows the proliferation of D in first stage. We should find the answer to this question in studying the innate immunity in the pityriasis folliculorum, where D proliferates a lot without any clinical immune reaction of the host: the immune defect is isolated there, not masked by the secondary immune reaction observed in PPR. So, this condition appears as a 'missing link' in the understanding of rosacea.

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