Review

Immunology of Dermatophytosis

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Abstract

Resistance to dermatophyte fungi depends on both non-specific and specific immunological mechanisms. Amongst the former epidermal proliferation, unsaturated transferrin and fatty acids in sebum play major roles. In addition activated phagocytic cells destroy dermatophyte fungi in vitro both intra- and extra-cellularly. In experimental murine dermatophytosis immunity is transferred to naive animals with lymphocytes bearing the Thy-1,2 phenotype. In man after experimental dermatophyte infection there is evidence that activation of T cell mediated immune responses correlates with recovery. By contrast in certain common dermatophyte infections in man there is evidence of poor T lymphocyte mediated responses (lymphocyte blastogenesis, DTH) to specific fungal antigens. An extracellularly expressed inhibitory factor produced by Trichophyton rubrum and other dermatophytes can suppress B and T lymphocyte blastogenesis in vitro. Circulating dermatophyte antigen is present in serum of infected patients and there is evidence of failure of expression of certain activation markers such as ICAM-1 on keratinocytes from chronically infected patients. These findings suggest that immunomodulation due to the presence of factor(s) derived from dermatophytes may play a role in determining the course of infection in some patients.

Key words: dermatophyte, immunity, antigen, epidermis

Introduction

The mechanisms of host resistance to organisms that infect the epidermis are necessarily different to those deployed against infections in other sites. This is not due, as originally thought, to some intrinsic lack of effective cells dedicated to immunological surveillance. Rather specific afferent and efferent pathways for immunologically mediated responses have evolved in the epidermis. Reception and processing of foreign antigens is largely performed by modified dendritic cells, Langerhans cells, as well as other cellular elements of the skin. Far from playing a passive role in the development of the immune response keratinocytes can be activated to express class II antigens and to release cytokines as well as other inflammatory mediators. Examples include tumour necrosis factors (TNFs) and interleukins 1 and 8 (IL-1, IL-8). They also express other factors involved in the development of an inflammatory responses such as the adhesion molecule, ICAM-1. In addition this network communicates actively with other arms of the immunological system including as B and T lymphocytes and neutrophils.

The importance of following these changes which develop in response to a foreign antigen is that the epidermis is “switched on”, a feature which helps localize the need for effector cells at one particular skin site and where it
is possible to reactivate the system at some later event\(^1\).

Perhaps the least well understood aspect of this epidermal activity is the means by which antigen reception is translated into an effector response. It is clear that certain effector mechanisms such as the migration of polymorphonuclear leucocytes into an area or cytokine regulated increased epidermal cell turnover may act to remove foreign substances including microorganisms from the skin surface. The effectiveness of such mechanisms in epidermal infection are not known or indeed whether efficacy varies from site to site on the skin surface. However it is possible that there are areas on the surface where immunological defence is less complete than others.

Likewise it is necessary to explain why the presence of certain organisms such as coryneform bacteria or *Pityrosporum* yeasts, which form part of the commensal skin flora, is generally tolerated; whereas in the case of others, including the dermatophyte fungi, an inflammatory response of varying degrees of efficiency is elicited. Is this due to products originating from the organisms and, if so, what constitutes the difference between a stimulus which is perceived by the immunological receptor system as being worthy of eliciting a response? While there is a range of different immunological receptor and effector mechanisms, the persistence of certain skin infections such as human papilloma virus infections and the fungal infections, pityriasis versicolor and dermatophytosis, would suggest that the process of elimination may be modified by local or systemic factors.

This paper is concerned with defence against one group of infections which involve the epidermis, the dermatophytoses, in which a range of different clinical and host inflammatory responses is seen. At one end of this spectrum of responsiveness there are dermatophyte infections which are self limited, elicit an inflammatory reaction and which generally respond to minimal therapy and do not recur\(^2\). Zoophilic dermatophytosis, infections of animal origin in man, are generally highly inflammatory and produce lasting immunity. For instance, second infections with the cattle ringworm, *Trichophyton verrucosum*, are rare\(^3\). By contrast human anthropophilic dermatophytoses are becoming increasingly common and are often persistent, sometimes despite therapy\(^4\). Relapse after treatment is also frequent. Examples include *T. rubrum* infections affecting the feet, *T. tonsurans* in the scalp\(^5\) and *T. concentricum*\(^6\) which causes extensive tinea corporis in remote areas of the humid tropics. Persistence of these infections is common and dermatophytosis accounts for 5–8% of new outpatient referrals in skin disease in the UK, up to 20% in the tropics. In certain occupational groups persistent dermatophyte infections are equally problematic. In the UK coal mining industry 30–35% of miners have chronic foot infections leading to moderate disability as well as significant economic loss through illness and industrial compensation\(^7\).

### Adherence

Dermatophyte fungi generally do not penetrate further than the granular layer of the stratum corneum. The first phase of attack consists of adherence between the fungal cell and the keratinocyte\(^8\). This has been shown to be a rate limited adhesion process in which the invading arthrospore is attached over a 2–3 hour period before germination. The process is accompanied by changes such as swelling of arthrospores. Experiments to elucidate the mechanisms of this adherence phenomenon in dermatophyte infections have been limited by the difficulty of producing single arthrospores rather than using microconidia and the fact that in life adherence between human keratinocytes carrying the fungi is the most likely initiation of attack. The process is easily inhibited by low drug concentrations. Penetration of the dermatophytes through this layer is followed by localization of the invasion to the outer stratum corneum\(^9\). In experimentally infected mice this process may well be limited
by the rapid recruitment of neutrophils to the site of attack\textsuperscript{10}, however in man we have no evidence of early mobilization of neutrophils in dermatophytosis, neutrophil accumulation being a feature of infections in which fungi penetrate the hair follicle\textsuperscript{11}.

Skin invasion by dermatophytes involves the production of proteases which are inducible in the presence of amino acid residues\textsuperscript{12}. While at least three low molecular weight proteases have been isolated from \textit{T. mentagrophytes}\textsuperscript{13}, a number of different proteases have been described for \textit{T. rubrum}. These range from 34, 77 and 105 kD moieties. In addition this dermatophyte produces a secreted metalloprotease with a molecular weight of c 200 kD which shows specificity for collagen and elastin (Lambkin, Hamilton and Hay- unpublished data).

**Immunity to infection**

Human dermatophyte infections can be classified as acute or chronic; acute infections in man are often caused by zoophilic fungi, chronic by anthropophilic organisms. Defence against dermatophytes depends on the activation of both immune and non-immune mechanisms. The principle pathways are unsaturated transferrin, migration of polymorphonuclear leucocytes into the area of infection and T lymphocyte activation\textsuperscript{2}. No work has identified an iron receptor in dermatophyte fungi, although siderophores are known in a variety of plant pathogens such as \textit{Alternaria} species. Unfortunately there has been little work on the so called nonspecific mechanisms of defence against dermatophytosis. Generally the fungi are inhibited by a number of different ways. Unsaturated transferrin inhibits the growth of dermatophytes by a direct mechanism involving binding to the fungal membrane\textsuperscript{14}. The speed of growth of the epidermis is also increased in human dermatophytosis; a similar phenomenon has been shown in experimental candidosis in guinea pigs\textsuperscript{2} and experimental dermatophytosis in mice. In these experimental infections increased epidermal proliferation occurs early in the infection, within 48 hours in mice, in other words before classical immunological activation can have occurred. Likewise increased proliferation can be seen in guinea pig skin infected by dermatophytes after engraftment onto un mice\textsuperscript{15}, showing once again that the process does not rely on an intact T lymphocyte system. There is an acceleration of the proliferative response in the guinea pig \textit{Candida} model after 10 days suggesting that at this stage there may be some amplification of the proliferative response by immunological mechanisms.

Killing of dermatophytes by both murine and human neutrophils and macrophages can be demonstrated. The chief effector cells which affect dermatophyte fungi are neutrophils (PMN) and, to a lesser extent, macrophages\textsuperscript{16}. The former are important components of the histological response to hair follicle invasion in man and animals. Human PMN have been shown to destroy up to 60\% of \textit{T. rubrum} and \textit{T. quinckeanum} germlings within two hours; macrophages kill up to 20\% in a similar time. PMN killing is enhanced by concanavalin-A or phorbol myristate acetate\textsuperscript{17}. The effects of PMN's have been reproduced by an \textit{in vitro} system using lactoperoxidase, hydrogen peroxidase and potassium iodide and can be abrogated with catalase, superoxide dismutase and histidine which are known scavengers of hydrogen peroxide, superoxide anion and singlet oxygen respectively\textsuperscript{17}. Killing can proceed even in the absence of ingestion of fungal hyphae by neutrophils. The existence of a second type of phagocyte mediated defence as occurs with \textit{Candida} via a peptide mechanism for dermatophytes has not been discovered. \textit{Trichophyton rubrum} produces a catalase which may affect the outcome of phagocyte mediated defence\textsuperscript{16}.

Neutrophils are attracted to the site of infection both by production of epidermally derived chemotactic factors such as leukotriene derivatives and also by certain dermatophyte cell wall antigens which activate the alternate pathway of complement\textsuperscript{18,19}. 
Susceptibility and immunomodulation

Previous studies have occasionally revealed a number of underlying diseases in patients with dermatophytosis notably hereditary palmoplantar keratoderma and Raynaud's phenomenon. A high proportion, over 40% in some surveys, are atopic on personal or family history. Patients with chronic mucocutaneous candidosis (CMC) are also particularly susceptible to widespread intractable dermatophytosis as well as candidosis; human immunodeficiency virus (HIV) infected individuals may also have chronic ringworm. These studies suggest that patients with impairment of immunological defence mechanisms, in particular those affecting T lymphocyte function are prone to chronic infection. However in all these examples whereas the clinical expression of infection may be modified by the patients' underlying condition the prevalence of dermatophytosis in compromised patients is not significantly different to that seen in healthy subjects. In AIDS patients the prevalence of infection is no higher than in members of "at risk" groups without HIV infection. Also, with the exception of atopy, underlying disease is not seen in the majority of patients with dermatophytosis. With infections due to C. albicans the picture is more confusing as two main groups of predisposed subjects are affected by this opportunistic pathogen—those with abnormalities of neutrophil or T lymphocyte function. However, individuals in the latter category, including patients with CMC and AIDS, usually have chronic cutaneous or oropharyngeal rather than systemic infections. Patients with CMC are also susceptible to dermatophytosis.

In experimental murine infections transfer of T lymphocytes bearing the Thy-1 helper phenotype is the key event in determining immunity. During primary infection in mice there is evidence of polyclonal suppression of lymphocyte activation during the phase of activation of lymphocytes reactive to dermatophyte antigens. Immunity to infection can be transferred to irradiated naive animals with lymphocytes bearing the Thy-1 phenotype, but not Ly-2.2. Transfer of antibody will also not convey resistance on recipients. In man there is a correlation between inflammatory responses, T lymphocyte blastogenesis and recovery. Patients with persistent foot infections (T. rubrum) or tinea corporis (T. concentricum) have reduced levels of lymphocyte blastogenesis or cytokine (e.g. migratory inhibitory factor) production in response to dermatophyte antigen. In chronic infections caused by T. rubrum, compared to those infections due to T. mentagrophytes for instance, there is weak lymphocyte transformation, inflammatory reactions and relapse or persistence of infection is common. In less than 15% of cases is there evidence of underlying disease affecting the immune system to explain these findings. In the rare disseminated forms of dermatophytosis where there is involvement of internal organs there may also be evidence of disturbance of immune (T lymphocyte) function. By contrast is most dermatophyte infections B lymphocyte responses are strikingly normal.

In addition to producing enzymes, oligosaccharides from glycoproteins present in dermatophytes are able to interfere with both T and B lymphocyte activation. This is a reversible process in vitro, but preexposure of lymphocytes to dermatophyte inhibitory factor or DIF will prevent proliferation of T cells in response to mitogens such as PHA as well as dermatophyte antigens. The fungal components which are most closely identified with host resistance via T cell activation are glycopeptides. In extensive or chronic dermatophytosis circulating antigen can be identified in serum. Skin and nail pathogens, therefore, contain the elements for receptor binding to host cells, penetration via enzyme production as well as both the elicitation and suppression of immune responses.

While the effector paths described previously provide potential methods of defence, the persistence of infection in many apparently
healthy individuals suggests that they are either ineffective or inoperative in some patients. It has been shown that some patients with persistent dermatophytosis have defective lymphocyte blastogenesis to T-cell mitogens and dermatophyte antigen and that this can be reversed either by substituting heterologous (foetal calf) for autologous serum or after successful antifungal treatment. A similar finding is seen with neutrophil responses. This suggests that an inhibitory factor(s) is present in serum and is evidence that the inhibition of lymphocyte blastogenesis by dermatophyte components may play a role in immunomodulation. The dermatophyte antigen, found in serum from infected patients, has been identified employing an immuno-radiometric assay using the mouse antidermatophyte IgM monoclonal TQ-1. Antigen derived from Trichophyton species containing TQ-1 reactive epitopes increases susceptibility of Balb/c mice to dermatophyte infection and interferes with T-cell mediated immunity (Calderon and Hay unpublished data). TQ-1 antibody reacts with phosphoryl-choline (PC) and immuno-reactivity can be abrogated by pre-treatment with PC. Phosphoryl-choline hapten is found in other parasites including filaria and also affects expression of immune responses in these infections. The other different factor identified as being part of the fungal cell wall is a high molecular weight glycoprotein. It is this that causes reversible inhibition of lymphocyte blastogenesis at certain concentrations. It is possible that a mannose component such as has been described in C. albicans may be responsible.

In vivo correlation

The possibility that dermatophytes interfere with the process of immunological activation in the skin is supported by observations on the presence of active molecules such as adhesion molecules in biopsies from chronically infected skin. Normally immunophenotypic techniques can demonstrate the presence of large numbers of effector lymphocytes in the vicinity of the infection. Work has now shown that the dermal infiltrate mainly contain cells which were Leu2a positive (viz T helper cells). Conversely few expressed CD-8 (T suppressor markers). HLA-DR was strongly expressed and most biopsies showed Langerhans cells using a variety of different cell markers. Despite the presence of an infiltrate adhesion molecules, such as ICAM-1, were poorly expressed in epidermis. This may reflect suppression of the production of these substances in the epidermis despite intact cell mediated immunity. Suppression of this aspect of immune activation by a dermatophyte inhibitory factor (DIF) produced by fungi in situ is a possible explanation, which may account for the success of dermatophytes such as T. rubrum in causing persistent infections.

Conclusions

Although much is known about the immunological response in human dermatophytosis there are still many loose ends, particularly the relationship between atopy and infection. There is evidence from a number of studies, described above, that atopic subjects are more susceptible to persistent infections, particularly if they are not involved in occupations where exposure to infection occurs frequently. Patients with persistent infection due to T. rubrum or T. concentricum are more likely to have positive immediate type immune responses to skin testing with trichophytin and raised specific IgE. It has also been shown that this occurs with T. rubrum infections in other sites such as the groin. It is not clear though whether it develops because of some defect in T cell responsiveness seen in atopic individuals or whether the production of specific IgE and consequent release of mediators such as histamine affect the expression of an effective immunological response. Whatever the reason the association between atopics and an increased tendency to superficial infection by bacteria, fungi and certain viruses is now well established.

The evidence put forward in this paper
suggests that during the course of dermatophytosis one reason for failure of cellular immunity and, subsequently, the emergence of chronic infection may be modification of the host’s immune response by fungal antigen. At present many studies of immunoregulation in skin have concentrated on diseases such as irritant dermatitis and psoriasis. However, a closer understanding of the pathogenesis of infections such as dermatophytosis may provide a new model for furthering our understanding of the means whereby immunological signals appear to be ignored by the immune system. With C. albicans other antigens have been found to mediate immunosuppressive responses. For instance, a glycoprotein extracted with ethylene-diamine suppresses transformation of murine splenic lymphocytes to a range of mitogens and cell wall mannann can elicit a similar response which is concentration dependent. Antigen mediated regulatory mechanisms seen with dermatophytes are therefore likely to be shared by other human fungal pathogens and possibly other organisms as well.

References


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