Natural Killer Cells in Psoriasis

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Abstract
Psoriasis is one of the most common immune-mediated disorders. There is evidence that it is mediated by Th1 and, more recently, Th17 cells. The cytokine pattern, particularly the dominance of TNF-α, implicates the innate immune system in psoriasis pathogenesis. Of the many components of the innate immune system known to be involved in psoriatic lesions, natural killer and natural killer T cells appear to have a unique role. We review the evidence supporting a role for natural killer cells in psoriasis.

Introduction
Psoriasis is a chronic inflammatory skin condition affecting 1–3% of the population (fig. 1) [1, 2]. It is now recognised as one of the most common immune-mediated inflammatory disorders [3]. Approximately 15% of patients with psoriasis develop psoriatic arthritis, a seronegative spondylarthropathy [2]. Patients with severe psoriasis also have an increased incidence of cardiovascular disease, hypertension, diabetes and obesity in addition to their skin and joint disease with resultant shortening of life [2].

Psoriasis is characterised by epidermal hyperplasia, angiogenesis and an influx of immunocytes into affected skin [3]. The exact trigger for the induction of a psoriasis plaque is unknown and several mechanisms have been proposed: The cathelicidin LL37 which is over-expressed in psoriatic skin binds to self-DNA, converting it to a potent trigger of plasmacytoid dendritic cells (DCs), and this may break innate self-tolerance and trigger the inflammatory cascade in psoriasis [4]. Streptococcal infection is a well-established stimulus of psoriasis. It is believed that the M protein of the bacterial cell wall may exhibit molecular mimicry of keratin 17; again this may...
provoke a reaction directed at self-proteins and trigger psoriasis [5].

The infiltrating immune cells produce inflammatory cytokines TNF-α and IFN-γ which can induce keratinocytes to produce adhesion molecules such as ICAM-1, costimulatory molecules CD40 and MHC II molecules further enhancing immune cell and keratinocyte interactions [3]. TNF-α also induces keratinocytes to produce VEGF causing endothelial cell proliferation and increased recruitment of lymphocytes perpetuating plaque formation.

**Current Therapies for Psoriasis Target T Lymphocytes**

Psoriatic plaques are dominated by the presence of Th1 and Th17 lymphocytes, macrophages, natural killer (NK) cells, and neutrophils with few B cells [6, 7]. The efficacy of alefacept [a fusion protein targeting leukocyte functioning antigen-3, LFA-3, which induces apoptosis of memory effector (activated) T cells in psoriasis] and efaluzimab (a monoclonal antibody to LFA-1), which also targets memory T cells in psoriasis, substantiate the view that T cells may have a primary pathogenic role [8, 9]. In addition, the efficacy of the fusion protein DAB 389-IL-2 (comprising IL-2 and fragments of diphtheria toxin), which is selective for activated T lymphocytes in psoriasis, suggesting T cells are critical for psoriasis pathogenesis [10, 11]. In contrast rituximab which targets B lymphocytes is not effective in treating the disease [12].

Th17 cells have been identified as being important in psoriasis pathogenesis with the identification of discrete subsets of Th17 cells in psoriatic plaques [13, 14]. IL-17 receptors are expressed on all cell types and are responsible for initiating inflammatory cytokine expression by the Jak/Stat pathway [15]. This has led to the development of a new treatment targeting Th17 cells via the IL-12 and IL-23 pathway. Ustekinumab, a monoclonal antibody directed against the shared p40 subunit of IL-12 and IL-23 is highly effective in the treatment of psoriasis [16–18].

**Therapies Targeting the Innate Immune System in Psoriasis Are More Effective**

The crucial role of TNF-α in plaque induction and the fact that TNF inhibitors appear to be more effective therapies than T cell-targeted therapies suggest that innate immune cells are very important in the pathogenesis of psoriasis (table 1) [19]. Alefacept and efalizumab are estimated to be effective in 30% of patients, whereas the TNF inhibitors infliximab and etanercept are estimated to be up to 90 and 50% effective, respectively.

Ciclosporin, a calcineurin inhibitor is one of the most effective treatments for psoriasis and is used to treat acute flares of disease. It has hitherto been considered to act only against T cells by inhibiting nuclear factors of activated T cells [20]. Nuclear factors of activated T cells are involved in the inducible expression of numerous genes involved in cytokine synthesis, particularly IL-2 [21]. In fact, ciclosporin targets calcineurin which is critical for cytokine production by all cell types. Ciclosporin therefore modifies the function of any cytokine-secreting cell including NK cells [20]. In vitro, ciclosporin results in dose-dependent reduction in cytokine release as well as reduction in IL-2- and IL-15-induced NK cell expansion.
NK cells are activated by IFN-α released by DCs and TNF-α upon stimulation of TLRs, usually endogenous TLR3, 7 or 9 which are activated by viral nucleic acids [28]. Once activated, NK cells serve to contain viral infections and often clear them without activating the adaptive immune response. They are also effective producers of large amounts of cytokines, in particular IL-4 and IFN-γ, and when activated by DCs, both cell types act synergistically to shape the adaptive immune response. NK cells are also capable of producing cathelicidins and β-defensins, antimicrobial peptides known to be over-expressed in psoriatic skin [29, 30]. Once in the skin, NK cells will produce IFN-γ which has been shown to be a highly effective promoter of Th17 cell trafficking to the skin in psoriasis [31].

NK-T cells are lymphocytes that express NK receptors, but unlike NK cells, also express T cell markers including CD3, TCR α and β [32, 33]. NK-T cells (like NK cells) may be cytotoxic and/or immunoregulatory. They are capable of significantly more cytokine production than NK cells, including secretion of IL-4, IL-5, IL-10, IL-17 and IFN-γ. A subgroup of NK-T cells known as invariant NK-T cells also express the restricted T cell receptor Vα24Vβ11. They are activated upon recognition of a glycolipid antigen presented by the CD1d molecule. This non-classical, antigen-presenting molecule has been found to be over-expressed on keratinocytes in psoriatic plaques [34]. In many respects, the immune response pattern in psoriasis is similar to that involved in viral infections and a viral aetiology has been proposed as the initial trigger of autoimmune inflammation in psoriasis [35]. Credence has been lent to this hypothesis by recent work demonstrating that single-strand RNA used to mimic viruses had the ability to stimulate DCs to express TLR7 and 8 in psoriasis [36]. These stimulated DCs produced IL-12p70, IL-1β, IL-6 and TNF-α, and were capable of stimulating naive T cells.

Table 2. Characteristics of NK cells in psoriasis

<table>
<thead>
<tr>
<th>Circulating NK cells</th>
<th>Reduced numbers of circulating NK cells [37]</th>
<th>Altered phenotype of circulating NK cells with upregulation of Fas and downregulation of CD94/NKG2A [70]</th>
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The innate immune system in psoriasis

Innate immune cells capable of producing TNF-α and IFN-γ in psoriasis plaques are NK cells, NK-T cells, DCs, neutrophils and macrophages [22]. Neutrophils accumulate in a perivascular fashion in early psoriasis lesions and then migrate to the epidermis where their collection manifests as pustules. DCs are increased in the dermis of psoriasis lesions, where they express markers of activation such as CD80, CD83 and CD86. Their numbers are augmented by inflammatory epidermal DCs and plasmacytoid DCs. DCs in psoriasis plaques appear to behave abnormally with decreased ability to migrate to normal stimuli [23]. The development of a new murine model of psoriasis (a CD18 hypomorphic mouse model) has ascribed a critical role to macrophages in the development of psoriasis [24].

Non-cellular components of the innate immune system identified in psoriasis include pro-inflammatory cytokines, altered patterns of Toll-like receptors (TLRs) on keratinocytes, heat shock proteins, anti-microbial peptides and C-type lectins [22]. TLR1, TLR2 and TLR5 have been found on psoriatic keratinocytes compared to uninvolved and normal skin. These cells also display differential expression with TLR5 down-regulated in active lesions. Heat shock proteins 27, 60 and 70 along with their ligands are over-expressed in psoriatic skin [25]. Human β-defensins 2 and 3, as well as cathelicidin LL37 are all over-expressed in psoriatic skin [26].

NK Cells in Psoriasis

NK and NK-T cells are particularly important producers of inflammatory cytokines, suggesting that these cells may play a critical role in causing psoriatic lesions. NK cells are key components of the innate immune response to tumour growth and viral infection [27].

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plaques, cells bearing NK receptors (CD94 and CD161) were found in lesional skin [38]. A second study using immunohistochemical techniques found that the cellular infiltrate in acute psoriatic plaques includes 5–8% NK cells, mostly localized in the mid and papillary dermis. NK lymphocytes isolated from biopsy specimens of psoriatic plaques showed a CD56brightCD16−CD158b− phenotype and released abundant IFN-γ upon stimulation [39].

In a separate study, tissue-infiltrating T lymphocytes expressing NK inhibitory receptors CD158b, CD94 and NKG2A were found in psoriatic lesions in significantly greater amounts than patients who did not have psoriasis [40].

**NK Cells Are Decreased in the Peripheral Blood of Patients with Psoriasis**

In contrast to the finding of increased NK cells in psoriatic plaques, several studies have documented decreased levels and decreased activity of NK and NK-T cells in the peripheral blood of patients with psoriasis. In an early study, the capacity of peripheral NK cells to kill a K562 tumour line was found to be decreased in 45 patients with psoriasis [41]. The numbers of circulating NK cells were significantly reduced in 14 patients with psoriasis compared to healthy controls [42]. There were significantly fewer cells expressing the NK cell markers CD16, CD56, CD94 and CD158a in patients with psoriasis compared with normal controls. A recent study has demonstrated altered phenotype of circulating NK cells in psoriasis of recent onset with up-regulation of Fas and down-regulation of the inhibitory receptors CD94/NKG2A [40].

The data regarding circulating numbers of NK-T cells is somewhat conflicting. In one study, NK-T cells (CD3+ CD56+), T cell (CD3+) and activated lymphocytes (CD69+) cells were not significantly different between patients with psoriasis and controls [42]. In a second study of 15 patients with psoriasis, peripheral NK-T cells significantly decreased compared to healthy controls [43]. After successful treatment to clear psoriasis, populations of circulating NK-T cells were significantly increased but did not reach values found in healthy controls. Invariant NK-T cells were also found to be decreased in the peripheral blood of 27 patients with psoriasis [44].

It is hypothesized that decreased levels of circulating NK and NK-T cells may be due to early activation by an as yet unknown stimulus followed by accelerated apoptosis. NK and NK-T cells are decreased in other Th1-mediated diseases such as rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis and insulin-dependent diabetes [45–49]. NK cells may act as a source of Th2 cytokines, reduced levels of which favour production of Th1 cytokines and resultant autoimmune activity [49]. In particular, NK cells can act as regulatory cells controlling the development of peripheral tolerance and modulation of immune responses. Dysfunction of NK-T and NK cells is observed in other T cell-mediated autoimmune diseases, the most studied of which has been type 1 diabetes [46, 47]. In non-obese murine models of type 1 diabetes, NK-T function and numbers were reduced. When NK-T cells were enriched, the diabetic phenotype was ameliorated [49, 50].

It is also possible that the reduction of circulating numbers of NK cells may be due to homing of NK cells to skin. It is still unclear whether the decrease is secondary to their consumption in a chronic inflammatory process or whether the decrease represents the sequestration of the cells in psoriatic lesions. The defect may in fact be a combination of both consumption and sequestration.

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<th>Table 3. Studies of NK and NK-T cells in psoriatic lesions</th>
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<tr>
<td>[37] Immunohistochemical study demonstrating increased numbers of NK and NK-T cells in psoriatic plaques of 10 patients compared to controls</td>
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<td>[38] Immunohistochemical study demonstrating the presence of NK cells in psoriatic plaques of 8 patients</td>
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<td>[39] Immunohistochemical study of 10 patients with psoriasis showing accumulation of NK and NK-T cells in psoriatic skin</td>
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<td>[40] Analysis by flow cytometry of T cells in psoriatic plaques in five patients indicating infiltration of CD 158b, CD 94 and CD NKG2A positive cells</td>
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<td>[71] Immunohistochemical study of psoriatic plaques in 7 patients indicating the presence of invariant NK-T cells</td>
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<td>[34] Immunohistochemical study of psoriatic plaques from 18 patients confirming the presence of invariant NK-T cells and over-expression of CD1d on keratinocytes</td>
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**Immunogenetic Influences: HLA-Cw*0602 Allele and Killer Cell Immunoglobulin-Like Receptors in Psoriasis**

NK cell function is regulated by a series of cell surface receptors known as killer cell immunoglobulin-like receptors (KIRs) [51]. These receptors interact with HLA
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Table 4. Studies of NK and NK-T cells in peripheral blood

<table>
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<tr>
<th>Study Description</th>
<th>Results</th>
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<tr>
<td>Flow-cytometric study of the cytotoxic ability of NK cells from 45 patients with</td>
<td>NK cells from psoriatic donors were injected into autologous normal skin, resulting in the development of classic psoriasis plaques.</td>
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<td>psoriasis showing decreased cytotoxicity in psoriasis</td>
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<tr>
<td>Flow-cytometric study of 14 patients with psoriasis showing lower numbers of NK</td>
<td>Injection of NK cells from psoriatic donors into autologous normal skin did not induce psoriasis.</td>
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<td>cells but normal numbers of NK-T cells</td>
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<tr>
<td>Flow-cytometric study of 15 patients with psoriasis with lower levels of</td>
<td>The increase in KIR2DS1 has also been observed in psoriatic arthritis, another HLA-Cw6-associated disease.</td>
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<td>circulating NK-T cells and their failure to normalise after treatment</td>
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<tr>
<td>Flow-cytometric study showing decreased circulating Vγ24 T cells in 27 patients</td>
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<td>with psoriasis showing altered phenotype of NK cells with down-regulating of CD94</td>
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<td>/NKG2A and up-regulation of Fas</td>
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<td>Flow-cytometric study of 15 patients with new onset psoriasis</td>
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Class I antigen and respond to changes in HLA class I antigen on cells of the body. Some KIRs are activatory while others are inhibitory for HLA class I antigen. KIR expression is controlled by up to 16 genes on chromosome 19q13.4 in any individual and these genes are highly polymorphic.

Psoriasis is strongly associated with the HLA-Cw*0602 allele encoding the HLA-Cw6 molecule. This molecule is recognized by the inhibitory receptor KIR2DL1 and the activatory receptor KIR2DS1 expressed on NK cells. In a recent genome-wide association study (Genetic Association Information Network) HLA-Cw*0602 was found to associate with psoriasis (p < 10^-56) [52]. In the most recent genome-wide association scan, this finding was confirmed. HLA-Cw*0602 was again found to be associated with psoriasis (p = 10^-214) [53]. This is particularly true of type I or early-onset psoriasis (onset prior to 35 years of age).

Genetic polymorphisms of KIRs appear to confer susceptibility to psoriasis in some populations. In 76 Japanese patients, the frequencies of KIR2DS1 and KIR2DL5 were significantly increased in psoriasis cases compared with controls [54]. KIR2DS1 was also found in 85% of 116 Polish patients with psoriasis compared to 55% of controls [55]. KIR genes did not, however, confer susceptibility to psoriasis in a Chinese cohort [56]. Almost half of the 178 patients included in this study had late-onset psoriasis. In this form of psoriasis, genetic susceptibility seems to be less important in psoriasis pathogenesis than early-onset disease. In Brazilian patients, an association of KIR2DS1 with Cw*0602+ in 26.5% of psoriasis vulgaris patients was observed, while it was present in only 5.4% of controls [57].

The increase in KIR2DS1 has also been observed in psoriatic arthritis, another HLA-Cw6-associated disease [58]. In a study of patients with psoriatic arthritis which looked at KIR genes that have HLA-C as their recognized ligand, an increased frequency of the activating KIR2DS1 was observed [59].

NK Cells Are Capable of Inducing Psoriasis in Animal Models

Strong evidence in support of a role for NK cells in psoriasis pathogenesis comes from work with animal models of psoriasis. Three groups have studied NK and NK-T cells in psoriasis using a severe combined immunodeficient (SCID) mouse xenograft model. Normal skin obtained from non-psoriatic donors, transplanted on SCID mice, developed into psoriasis after injection of peripheral blood-derived immunocytes from psoriatic donors [60]. The cells found in the 'graft-psoriasis' area expressed CD94, CD158a, CD158b and CD161, all surface markers expressed by NK cells. In a second experiment using the same SCID mouse-human skin graft model, NK cells from psoriatic donors were injected into autologous non-lesional psoriatic skin. This resulted in the development of classic psoriasis plaques [61]. NK cells from normal donors injected into autologous normal skin did not induce psoriasis.

Another group reproduced this experiment by grafting non-lesional skin from patients with psoriasis and skin from non-psoriatic donors onto an SCID mouse model [62]. Injection of NK cells from psoriatic donors into autologous non-lesional psoriatic skin resulted in classic psoriasis histology with a significant increase in epidermal thickness and proliferation. Injection of NK cells from normal donors into autologous normal skin did not induce the histology of psoriasis, but that of psoriasiform dermatitis. This was a non-specific reaction pattern.

In a different SCID mouse strain (AGR 129) devoid of T and B cells while also having immature NK cells with severely impaired cytotoxic activity, uninvolved skin from psoriatic donors was transplanted [63]. Using a skin explant model of both normal and psoriatic skin, resident T cells contributed to the development of psoriasis-like disease in the previously normal 'uninvolved' psoriasis donor skin. It has been proposed that the resident T cells in this model were NK-T cells. Bacterial superantigens

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were relatively potent inducers of IFN-γ and NK-T cells were observed in greater numbers in psoriatic skin [64].

Although these models provide strong mechanistic evidence for a role for NK cells and NK-T cells, uncertainty exists as to how well the skin eruption seen in experimental mice correlates with psoriatic plaques in humans. To date, it has proven difficult to develop an adequate animal model of psoriasis.

Conclusion

There is considerable circumstantial evidence to suggest that NK cells are key immunocytes in the pathogenesis of psoriasis. Immunohistochemical and flow cytometric studies have demonstrated the presence of NK cells in psoriatic plaques. Animal models provide strong evidence that NK cells may be necessary for the development of psoriatic lesions. These models are somewhat flawed due to uncertainty as to how representative the lesions observed are of psoriasis. Perhaps the strongest evidence comes from the strong genetic association between genes and single nucleotide polymorphisms of NK cell biology and psoriasis. To date, these remain the most important genetic associations for psoriasis.

Nevertheless, there are large gaps in our understanding of the exact role of NK cells in the initiation of psoriatic lesions. A primary question is whether their activation and migration to the skin is pivotal to the development of plaques or whether they represent a secondary phenomenon. Several of the more successful therapies for psoriasis are directed against cytokines (TNF inhibitors) or their production (ciclosporin). As NK cells are potent producers of inflammatory cytokines, it is likely that they are primary targets for these therapies. The possibility that NK and NK-T cells may represent more specific therapeutic targets highlights the need for further understanding of NK and NK-T cells in psoriasis.

References

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