Response of Murine and Normal Human Skin to Injection of Allogeneic Blood-Derived Psoriatic Immunocytes

Detection of T Cells Expressing Receptors Typically Present on Natural Killer Cells, Including CD94, CD158, and CD161

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Background: The genetic and immunological basis for psoriasis is unknown. Through the use of a severe combined immunodeficient mouse–human skin model, T cells have been shown to induce psoriasis, which points to a pathological role for such immunocompetent cells. During ongoing studies using this model, a previously overlooked subset of immunocytes expressing receptors typically present on natural killer (NK) cells was discovered, which may shed new light on the genetic susceptibility for psoriasis.

Observations: Immunocytes from a psoriatic patient were injected into engrafted allogeneic normal human skin and produced a psoriatic plaque. Moreover, the disturbed epidermal environment spread to induce a greater than 20-fold increase in thickness of adjacent mouse epidermis with prominent elongation of rete pegs. Thus, rather than observing the predicted graft-vs-host reaction in the allogeneic human or xenogeneic mouse skin, injection of psoriatic immunocytes triggered psoriasis. To explore a potential mechanism to explain the lack of cytopathic effect by psoriatic T cells, immunostaining to detect inhibitory receptors normally present on NK cells was performed. These receptors include surface molecules that can inhibit NK cell proliferation, cytokine release, and/or cytotoxicity (ie, killer cell inhibitory receptors [KIRs]), as well as those that may activate NK cell cytotoxicity (ie, killer cell activating receptors [KARs]). The blood-derived psoriatic immunocytes in the skin graft expressed CD94, CD158a, CD158b, NKB1, and CD161. Furthermore, both hyperplastic human and murine keratinocytes express the major histocompatibility complex (MHC) class I–like CD1d protein, which has been shown to be a specific ligand of T cells expressing CD161 and other NK cell–associated receptors.

Conclusions: Since several KIRs and KARs are known to recognize various class I MHC alleles, and because psoriasis inheritance and susceptibility has been linked to various class I MHC molecules, we propose a novel hypothesis in which the pathogenic T cells are postulated to express an assortment of KIRs and KARs. These interactions may produce direct activation without any exogenous antigen, and at the same time block the cytotoxic effector function of these activated immunocytes in this allogeneic and xenogeneic experimental setting. In addition, human T cells expressing CD161 may be capable of interacting with human and murine CD1d expressed by the epidermal keratinocytes. These unexpected findings demonstrate that psoriasis is an immunological disease in which pathogenic T cells rather than epidermal keratinocytes are of primary importance. Functional studies will determine if targeting this previously overlooked population of immunocytes with blocking reagents will generate a novel immunotherapeutic strategic pathway for psoriasis, and whether disease susceptibility and/or incidence patterns can be explained by genetic abnormalities involving these ligand–receptor interactions.

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SorIaSIS IS a common and enigmatic skin disease that involves the interplay of recruited blood-derived immunocompetent cells and endogenous dermal and epidermal cell types. Progress in defining the genetic susceptibility to the disease and the pathophysiology of psoriasis has been hampered by the lack of a suitable animal model. To investigate the pathological basis of psoriasis, we developed an animal model in which human skin is engrafted onto severe combined immunodeficient (SCID) mice and then injected with blood-derived superantigen-activated immunocytes.1 In the past, investigators focused on conventional T-cell subsets,2 but it is now apparent that the search for pathogenic T cells should include consideration of T cells that may express receptors typically present on natural killer (NK) cells (NKRs).

Natural killer cells express inhibitory receptors (KIRs) specific for major histocompatibility complex (MHC) class I antigens, thereby preventing NK cell–induced lysis of self MHC class I-bearing cells. Furthermore, both hyperplastic human and murine keratinocytes express the major histocompatibility complex (MHC) class I–like CD1d protein, which has been shown to be a specific ligand of T cells expressing CD161 and other NK cell–associated receptors.
PATIENTS, MATERIALS, AND METHODS

PATIENTS

Keratome samples on NN skin were obtained, after informed consent, from the healthy skin of 2 different patients who had no evidence of psoriasis and were otherwise healthy. In addition, a separate punch biopsy specimen from a chronic untreated plaque (PP skin) was obtained from the same patient with psoriasis who donated blood. Immuncytes were derived from heparinized blood from this patient, a 36-year-old woman with mild to moderate plaque-type psoriasis who was not undergoing any recent (past 6 months) or current treatment.

HUMAN SKIN/SCID MOUSE CHIMERA AND TISSUE PROCESSING

Human skin xenografts of skin from healthy individuals were orthotopically transplanted onto 7- to 8-week-old CB17-SCID mice (Taconic Farms Inc, Germantown, NY) following previously described procedures.1 Two to 3 weeks after transplantation, autologous or allogeneic immunocytes (2-4 × 10^6 cells) diluted in 300 µL of sterile phosphate-buffered saline solution were injected intradermally into the xenograft. Human skin/SCID mouse chimeras were killed within 2 to 3 weeks of the last intradermal injection. Biopsy specimens were mounted on gum tragacanth (Sigma Chemical Co, St Louis, Mo), snap frozen in liquid nitrogen–chilled isopentane, and stored at –80°C.

IMMUNOSTAINING

Cryostat sections of skin were acetone fixed and stained using either a highly sensitive avidin-biotin immunoperoxidase technique with 3-amino-4-ethylcarbazole to produce a positive-red reaction product as described,1 or an indirect immunofluorescence procedure was performed. For immunoperoxidase single-antigen staining, the following antibodies were used to detect their respective antigens: anti-CD3, CD4, CD8, CD57, and NKB1 (Becton-Dickinson, Mountain View, Calif); anti-CD94 (clone Hp-3B1), CD158a (clone EB6), CD158b (clone GL183), CD161 (clone 191B8), TIA-1, CD45RA, and monomorphic determinant of HLA-A, HLA-B, and HLA-C (Coulter Corporation, Miami, Fla). Anti-CD45RO, CD45RA, IL-15, CD56, antihuman Ly49, CD16, H2Dd, and CD1d (clone 3C11) were purchased from PharMingen, San Diego, Calif. Human CD1d was detected using clone NOR3.2 mAb (Biosource International, Camarillo, Calif).

Two-color immunofluorescence staining was performed using cryostat sections incubated with mouse anti-human monoclonal antibody against the following antigens: mouse antihuman CD94 (Coulter Corporation), mouse antihuman CD158a (Coulter Corporation); and either rabbit antihuman CD4 (Coulter Corporation) or goat antihuman CD8 (BDI Corporation, Newark, NJ) was used at 10 µg/mL final concentration for 30 minutes at room temperature. After washing in fluorescent antibody buffer (Difco, Detroit, Mich), a rhodamine-conjugated goat antimouse antibody (1:50 dilution; Biosource International), or fluorescein isothiocyanate–conjugated antirabbit antibody (Biosource International), or fluorescein isothiocyanate–conjugated swine antigoat (Biosource International) was added for 30 minutes at room temperature. For all these reactions, after the last wash in fluorescent antibody buffer, a mounting solution was applied (para-phenylenediamine in glycerol) prior to using a cover slip. All slides were examined and photographed using an Olympus (Tokyo, Japan) AX-80 microscope.

CELL CULTURE STUDIES

Immuncytes from normal volunteers and a patient with psoriasis were isolated from heparinized blood with the use of Ficoll-Hypaque (Pharmacia LKB Biotechnology Inc, Piscataway, NJ) density centrifugation. Samples of 1 to 2 × 10^6 peripheral blood mononuclear cells per milliliter were cultured on tissue culture dishes (Corning Glass Works, Corn ing, NY) in complete media containing 10% heat-inactivated autologous serum with or without 1 µg/mL each of staphylococcal enterotoxins B and C2 (Toxin Technologies, Sarasota, Fla) and 20 U/mL of human IL-2 (Boehringer Mannheim Biochemicals, Indianapolis, Ind) for 72 hours at 37°C in a humidified atmosphere containing 5% carbon dioxide as previously described.1

target cells.3 In addition, killer cell activating receptors (KARs) bearing some of the same features but with distinct intracellular signaling can also activate or promote NK cell–mediated lysis of MHC class I–bearing targets.4,6 Both KIRs and KARs originally characterized on NK cells can also be expressed by T cells.7 There is also a subpopulation of so-called NK-T cells expressing CD161 with distinctive phenotypic and functional characteristics.8,9 In contrast to KIRs/KARs, which recognize MHC class I molecules, CD161+ NK-T cells mediate reactions involving the nonclassical MHC–like molecule, CD1d.8,9

In humans, there are basically 2 distinct molecular groups of KIRs and KARs.1,5 One group possesses 2 (ie, CD158a and CD158b) or 3 (ie, CD158a, CD158b, and NKB1) immunoglobulin-like domains with specificities for either MHC class I molecules HLA-B or HLA-C. The second group are C-lectin–type receptors, CD94/NKG2, and CD158a with a broader recognition capability that includes, for CD94/NKG2, leader sequence peptides of a broad range of MHC class I alleles presented by HLA-E.7 Certain cytokines such as interleukin (IL) 2 and IL-15 can influence these surface receptors on superantigen-activated T-cell subsets.10,11 Interleukin 15–induced expression of both CD94 and NKG2A on CD8+ T cells and such phenotypic changes were accompanied by dramatic functional alteration with reduced cytotoxic activity by T cells induced to express these KIRs (ie, CD94/NKG2A). Interestingly, in NK cells when CD94 is coexpressed with NKG2C, but not NKG2A, or with truncated cytoplasmic domains of CD158a and CD158b, recognition of appropriate class I–bearing target cells leads to activation of the NK cell rather than an inhibitory response.6,12-14

As T cells bearing NKRs are capable of producing large amounts of cytokines15 that include gamma inter-
feron (IFN-γ), which by itself can cause psoriasis, and because of the role of NK-T cells in regulating autoimmune diseases, we explored their potential role in psoriasis, a skin disease believed to be an autoimmune disorder. Engrafted symptomless skin from subjects with psoriasis (PN skin) can be converted, using a novel SCID mouse–human skin model, to full-fledged psoriatic plaques (PP skin) by injection of superantigen-activated immunocytes. Not only could autologous immunocytes consistently induce psoriatic lesions (5 different subjects), injection of activated allogeneic blood-derived immunocytes from patients with psoriasis into engrafted skin from healthy individuals without psoriasis (NN skin) created full-fledged psoriatic lesions in 3 of 6 mismatched patient combinations examined. It was puzzling why activated allogeneic T cells would produce psoriasis rather than consistently provoking graft-vs-host disease; however, graft-vs-host disease was observed in 1 patient combination in this experimental setting. Moreover, a recent report demonstrated that a large percentage of intraepidermal CD8⁺ T cells in chronic psoriatic plaques contained TIA-1 (a marker for cytotoxic granules), but without any evidence of a cytotoxic effect on adjacent keratinocytes. Taken together, these results suggested that while activated CD8⁺ T cells are present in acutely generated as well as stable psoriatic plaques, lack of cytotoxic effects may involve participation of surface receptors on these T cells that suppress cytotoxicity upon activation.

Another reason to suspect involvement of T cells bearing NKRs in psoriasis was the discovery that pheno- typic conversion of mouse skin occurred adjacent to engrafted NN skin. As documented in this article, both murine and normal human skin concomitantly developed into a psoriatic plaque following injection of immunocytes from a patient with psoriasis. Once again, not only did psoriatic T cells fail to induce graft-vs-host disease in the allogeneic human skin (ie, no necrotic keratinocytes, basal vacuolization, or subepidermal clef formation), but also no evidence of graft-vs-host disease was observed in the xenogeneic mouse skin. Thus, it appeared that these allogeneic immunocytes bearing the class I and class I-like MHC receptors CD94, CD158a, CD158b, NKB1, and CD161 provoked a keratinocyte and endothelial cell hyperplastic response highly characteristic of psoriasis in both human and mouse skin. Since murine CD1d can be recognized by human NK-T cells, such molecular interactions may be involved in cross-talk between human immunocytes and murine keratinocytes. While CD1d has previously been detected only on intestinal epithelial cells, our current article provides the first documentation that epidermal keratinocytes also express CD1d.

**RESULTS**

Engrafted skin from healthy individuals injected with phosphate-buffered saline solution but no immunocytes had a normal epidermal thickness (mean ± SD rete length, 68 ± 14 µm) and a stratum corneum without parakeratosis, an intact granular cell layer, and rare CD3⁺ T cells in the upper dermis. A different NN skin sample after engraftment and injection of autologous activated immunocytes revealed no psoriasis in either the human or mouse skin (mean ± SD murine epidermal thickness, 23 ± 6 µm), and low numbers of scattered CD3⁺ T cells were present in both human and mouse dermis (data not shown). In contrast to these grafts, when NN skin was injected with allogeneic-activated immunocytes from a patient with psoriasis, there were clinical changes noted that included scaling, erythema, thickening, and an angiogenic tissue reaction (data not shown) as well as massive epidermal hyperplasia (Figure 1, A) (mean ± SD rete length for human and mouse skin, 593 ± 86 µm and 547 ± 92 µm, respectively). Interestingly, it was noted by routine light microscopy under immunohistochemical staining for human monomorphic HLA-A, HLA-B, and HLA-C determinants (Figure 1, A) that, while engrafted skin in the plaque had relatively uniformly increased epidermal thickness, approximately 40% of the plaque contained parakeratotic scale and loss of the granular cell layer (Figure 1, A, at left), while the other 60% of the plaque had no parakeratotic scale with increased and prominent granular cell layer (Figure 1, A, at right). The former portion represented human epidermis (Figure 1, A, at left), whereas the latter portion did not express these human class I MHC antigens (Figure 1, A, at right), suggesting it was murine epidermis.

In more than 3 years of examining a wide variety of injected human and murine skin samples engrafted onto CB17-SCID, ICR-SCID, and CB17-SCID/Beige strains of mice, we have never observed anything closely resembling this extent of epidermal thickening with such extensive and uniform elongation of rete pegs by mouse epidermis. Occasional murine class I MHC⁺ cells were present in dermis underlying the human epidermis; however, in those areas of epidermis with compacted stratum corneum and granular cell layer, the epidermis was diffusely and strongly positive for murine H-2D expression (Figure 1, B).

In the human skin portion of the plaque, human dermal and epidermal CD3⁺ T cells were present in a non-random fashion, similar to the appearance of autologous immunocytes and symptomless skin from subjects with psoriasis. Human CD4⁺ T cells were conspicuous in both the epidermal and dermal compartments, compared with CD8⁺ T cells, which were more conspicuous in the epidermis compared with the dermis (data not shown). Examination for TIA-1–positive cells revealed numerous intraepidermal and upper dermal immunocytes in the human skin graft (Figure 1, C), resembling psoriatic plaques directly obtained from patients. To determine if the lack of any cytotoxic effect (ie, no graft-vs-host disease) of these allogeneic CD4⁺ and CD8⁺ T cells in the engrafted human skin could be related to the presence of KIRs, the samples were stained to detect CD94, CD158a, CD158b, and NKB1. While less than 1% of peripheral blood-derived T cells express KIRs and/or KARs, numerous intraepidermal and dermal CD94⁺ immunocytes were identified in the human portion of the psoriatic lesion (Figure 1, D). There were approximately half as many immunocytes expressing CD158b, a quarter as many cells expressing NKB1, and fewer than 10% of cells expressing CD158a (data not shown). Also, only rare hu-
man NK cells (ie, CD57+) were present (fewer than 1%) in the psoriatic plaque. When the skin graft from healthy individuals injected with autologous immunocytes was examined for CD94 expression, only occasional CD94+ cells were present in the dermis (data not shown).

Examination of the murine portion of the skin lesion (selected from sites with no parakeratosis and prominent granular cell layer) revealed numerous human CD3+ T cells infiltrating dermis and epidermis. As was seen in the human portion of the skin graft, almost all of these infiltrating T cells in the murine skin were human CD45RO+ (ie, memory) with only rare CD45RA+ (ie, naive) T cells. Since IL-15 can induce CD94 expression on T cells,11 we explored its expression and found IL-15 primarily in upper dermal macrophagelike cells (data not shown). Numerous CD94 positive immunocytes were present in the epidermis and upper dermis of murine skin. There were also scattered NKB1+ immunocytes, and CD158b+ immunocytes, with only very rare CD158a+ immunocytes (data not shown). In addition to these human immunocytes infiltrating the mouse skin, scattered murine CD16+ immunocytes were present in mouse epidermis and dermis. In this murine CD16+ population of mononuclear cells, scattered murine cells expressing Ly49 were identified. Nonspecific staining using purified mouse immunoglobulin revealed no reactivity. Injection of psoriatic immunocytes into mouse skin devoid of any human skin xenografts revealed no substantial kerato-
cyte hyperplasia or angiogenic tissue response and no trafficking of human cells into mouse epidermis (data not shown). To determine which type of T cell was expressing CD94, 2-color immunofluorescence staining was performed. Examination of these double-labeled slides revealed that both CD4+ and CD8+ T cells included subsets that expressed CD94 (data not shown).

Based on these SCID mouse results, we also examined the biopsy specimen of a plaque directly removed from the same patient with psoriasis from whom immunocytes were obtained and observed the presence of numerous TIA-1–positive T cells in epidermis and dermis (data not shown), as well as scattered CD161+, CD94+, and CD158b+ immunocytes in the epidermis (Figure 2).

To explore the possibility that human CD161+ T cells were involved in the hyperplastic response of both murine and human epidermis via interaction with CD1d, the psoriatic plaque removed from the SCID mouse was immunostained to detect CD1d and CD161 (Figure 3). The human skin portion of the graft had CD1d expression decorating the plasma membrane of keratinocytes in the mid and upper levels of the epidermis (Figure 3, A), and the murine portion of the graft also had prominent CD1d expression of the keratinocyte plasma membranes producing a “chicken-wire” pattern (Figure 3, B). CD161+ immunocytes were present infiltrating both the human and the murine portions of the graft (Figure 3, C).

COMMENT

These unexpected results obtained using the SCID mouse–based animal model indicate that the primary defect in psoriasis is most likely related to the immunocyte rather than the keratinocyte. Thus, even skin obtained from normal healthy individuals can be phenotypically converted to psoriasis by transfer of immunocytes obtained from the blood of a patient with psoriasis. Previous studies1,24-27 that highlight the primary pathogenic role for the immune system in psoriasis also provide support for this conclusion. Not only can psoriatic T cells cause skin le-
sions in humans and mice, the current studies also indicate a potential role for KIRs and/or KARs and CD161 and/or CD1d in psoriasis. Indeed, immunostaining of a stable untreated plaque removed from the same patient with psoriasis who donated the blood revealed intraepidermal immunocytes that expressed CD94, CD158b, and CD161. Thus, T-cell subsets expressing KIRs and/or KARs such as CD94 and/or CD161 may play a previously overlooked role in psoriasis. Even though the overall number of T cells expressing CD94, CD158a, CD158b, NK1, and CD161 was less than 10% of the total intraepidermal T-cell population, they still may be an important component of the pathogenic T-cell population. This possibility is highlighted by findings in allergic contact dermatitis in which the frequency of pathogenic antigen-specific T cells is established to be less than 1%, and may even be less than 0.1% of total T cells.28

Based on this line of inquiry, we suggest a novel hypothesis that may explain the genetic basis and immunobiological pathway responsible for causing this skin disease emphasizing the potential role of KIRs and/or KARs expressed by pathogenic T cells. First we will consider the genetic analysis of psoriasis. Even though a specific gene has not been consistently identified, there seems to be a consensus that the MHC class I region of chromosome 6 holds the most promise for determining disease susceptibility and incidence.29,30 Current dogma has pointed to the ability of class I alleles to present foreign peptides to CD8+ T cells,31 and the finding that intraepidermal CD8+ T cells are of clonal origin supports such a notion.32 However, rather than considering the involvement of an exogenous antigen,33 we postulate that it may be the direct activation of T cells bearing receptors for MHC class I that triggers psoriasis.34

Human CD8+ T cells expressing NKRs for class I MHC molecules that represent oligoclonally or monoclonally expanded populations have been observed in peripheral blood.35 As one examines class I alleles frequently implicated in psoriasis from the KIR and KAR perspective, several correlations become apparent. Perhaps the most striking and intriguing correlation involves HLA-C. The specific region of this HLA molecule influencing KIR and KAR recognition has been determined to involve amino acid residues 77 and 80.35 Interestingly, if alanine is present at position 73 of HLA-C, there is a strong correlation to psoriasis.36 Thus, we postulate that certain alleles, or mutations in class I alleles or CD1d, may trigger T-cell activation by KAR-bearing or CD161+ autoreactive T cells that were not appropriately deleted during development in an analogous fashion as NK cells are triggered.37 Since CD94 and CD161 have a lectin domain, carbohydrate moieties attached to or bound by class I alleles or CD1d may also be important, and the appropriate carbohydrate-bearing molecule expressed by either normal human skin or by murine skin within the milieu of the relevant cytokine network28 may be able to trigger the direct activation of the KAR-bearing pathogenic T cell subset. The mechanism by which CD94 and other KIRs and/or KARs are up-regulated in this model system is unknown.10,11

A second component of this hypothesis is that since CD8+ T cells and NK cells are derived from a common precursor and can serve similar immunological functions,30 there may also be involvement of KIR-like molecules that suppress the cytopathic effector pathway.40 Many CD8+ T cells expressing TIA-1 are present but do not damage the surrounding allogeneic and xenogeneic keratinocytes. We postulate that the overall expression of KIRs and/or KARs37 on the pathogenic T cells in psoriasis is configured in such a way that the immunocytes are directly triggered by the aforementioned class I MHC receptors to release cytokines and cause psoriasis but do not damage or kill the keratinocytes. Clearly there are considerably more TIA-1+–positive cells than CD94+, NK1+, CD158a+, and CD158b+ cells, suggesting that other molecules may be present to inhibit cytotoxicity. We also cannot exclude the possibility that there is a peptide associated with the class I MHC molecules, but at least one report established that there is a peptide-independent recognition pathway.42

Besides classic class I MHC molecules, there are several other class I genes that may be relevant in this clinical setting.43-46 In addition, the MHC class I-like molecules such as CD1d may be involved in psoriasis. Compared with normal human skin, there is extensive overexpression of CD1d by the hyperplastic keratinocytes in psoriasis (B.J.N., unpublished data, 1998), as reflected by this engrafted human skin sample. Future studies are required to confirm the possibility that the human CD161+ immunocytes are capable of interacting with the murine epidermal keratinocyte CD1d in vivo, as has been observed in vitro.22 It will also be important to determine which (if any) glycolipids may be participating in the CD1d-mediated activation of CD161+ T cells, and whether the altered stratum corneum in psoriatic plaques is the source of such glycolipids.9,22,23

While we have been critical of other animal models of psoriasis for not possessing relevant histological features, the current results indicate that mouse epidermis can become significantly more hyperplastic than previously recognized with extremely elongated rete pegs.47 Interestingly, even with this degree of acutely induced keratinocyte hyperplasia, the granular cell layer in the murine skin graft was preserved. This histological feature also has implications for understanding the differentiation pathway operative in psoriasis.48 Since single injections of pathogenic immunocytes directly into SCID mouse dermis devoid of human skin does not provoke keratinocyte or endothelial cell hyperplasia, the striking murine response clearly required the presence of a disturbed human epidermal and dermal environment in adjacent skin. Functional studies are underway to elucidate the stimulus triggering KIR and KAR expression by pathogenic immunocytes in vivo, and to determine if blocking this novel pathway will prevent psoriasis. It is possible that psoriasis is so prevalent worldwide because these previously overlooked immunocytes with NKRs provide important innate-immune functions against infectious and neoplastic insults, which would explain why these conditions are less frequent in patients with psoriasis.30 Learning more about this subset of immunocytes may also be relevant in other cutaneous...
disorders. In conclusion, the SCID mouse–based xenotransplantation model involving human skin continues to provide new insights into the etiology and pathophysiology of psoriasis.

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