Melanocytes: Target Cells of an HLA-C*06:02–Restricted Autoimmune Response in Psoriasis

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HLA-C*06:02 is the main psoriasis risk allele. By the unbiased analysis of a Vα23S1/Vβ13S1 T-cell receptor from pathogenic psoriatic CD8⁺ T cells, we had recently proven that HLA-C*06:02 directs an autoimmune response against melanocytes through autoantigen presentation in psoriasis and identified ADAMTS5 as a melanocyte autoantigen. We concluded that psoriasis is based on a melanocyte-specific immune response and that HLA-C*06:02 may predispose to psoriasis via this newly identified autoimmune pathway. Understanding this pathway, however, requires more detailed explanation. It is based on the fact that an HLA class I-restricted autoreactive CD8⁺ T-cell response must be directed against a particular target cell type, because HLA class I molecules present peptide antigens generated from cytoplasmic (i.e., intracellular) proteins. This review summarizes the findings on the melanocyte-specific autoimmune response in the context of the immune mechanisms related to HLA function and T-cell receptor–antigen recognition. Identifying melanocytes as target cells of the psoriatic immune response now explains psoriasis as a primary autoimmune skin disease.

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INTRODUCTION

Psoriasis is a T-cell mediated disease that primarily affects the skin. The psoriatic immune response drives psoriatic inflammation through a complex cytokine pattern that is dominated by the signature cytokines IL-17A, IL-22, and IFN-γ (Lowes et al., 2008; Perera et al., 2012). Although the psoriatic cytokine network has been elucidated in detail, the mechanisms activating the psoriatic T-cell response have remained controversial. Marked oligoclonality of the T-cell populations within psoriatic skin lesions definitely indicated that T-cell activation is driven by antigens in situ (Chang et al., 1994; Diluvio et al., 2006; Menssen et al., 1995; Vollmer et al., 2001). However, the antigen specificity of the lesional psoriatic T-cell response was still in doubt, and T-cell activation was rather attributed to the stimulatory effects of unspecific innate immune mechanisms (Nestle et al., 2009). This perception is now outdated since we showed that HLA-C*06:02 directs an autoimmune response against melanocytes through autoantigen presentation (Arakawa et al., 2015).

THE ROLE OF HLA CLASS I MOLECULES IN IMMUNITY

HLA-C*06:02 is the major psoriasis risk allele

As part of a complex genetic predisposition, the HLA allele HLA-C*06:02 is the main gene for psoriasis risk (Nair et al., 2006; Tiilikainen et al., 1980; Zhou et al., 2016). It is located in psoriasis susceptibility locus 1 (i.e., PSORS1) within the major histocompatibility complex (MHC) on chromosome band 6p21.3 that carries 50% of psoriasis risk (Burden et al., 1998; Nair et al., 1997; Trembath et al., 1997). Outside the MHC, genome-wide association studies and more targeted candidate gene approaches have identified more than 40 single-nucleotide polymorphisms associated with psoriasis at a genome-wide significance level (Ellinghaus et al., 2010, 2012; Huffmeier et al., 2010; Liu et al., 2008; Nair et al., 2009; Sheng et al., 2014; Strange et al., 2010; Stuart et al., 2010; Sun et al., 2010; Tsolou et al., 2012, 2015; Yin et al., 2015; Zhang et al., 2009) that exert only modest individual effects and contribute an estimated 20% of psoriasis risk (Tsoi et al., 2012). According to the concordance rate in monozygotic twins, environmental and lifestyle factors and infections account for the remaining approximately 30% of disease risk (Grijpbroek et al., 2007).

The HLA class I association of psoriasis is an extraordinary disease feature: of the more than 12,000 HLA class I alleles described today (IPD-IMGT/HLA Database; Marsh et al., 2010), only three show major disease associations: HLA-B*27 with ankylosing spondylitis, HLA-B*51 with Behçet’s disease, and HLA-C*06:02 with psoriasis. This indicates that the HLA class I alleles have a particular role in disease manifestation. The polymorphisms of the HLA alleles result in variable peptide-binding grooves that determine the antigen peptide repertoires presented by the HLA molecules (Falk et al., 1991, 1994). Because different HLA molecules select different peptide repertoires (Rock et al., 2016; Vyas et al., 2008), presentation of select self-peptides by certain HLA molecules would be the most straightforward explanation for the HLA class I association. Because of a strong linkage disequilibrium of HLA class I alleles within the MHC and missing functional imputation, however, the actual pathogenic significance of HLA-C*06:02 in psoriasis remained

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elusive, although extensive sequencing of the HLA region in psoriasis patients clearly defined HLA-C*06:02 as the actual risk carrier (Nair et al., 2006; Zhou et al., 2016).

**HLA class I-restricted immune responses are directed against target cells**

Although the natural function of the proteins encoded by HLA alleles is antigen presentation to T cells, there are fundamental differences between HLA class I and II molecules in terms of the antigen source. HLA class II molecules are expressed on professional antigen-presenting cells. They mainly present peptide antigens from exogenous proteins that have been degraded in the endocytic pathway to CD4+ T cells (Neeffjes et al., 2011). Thus, they screen the environment for antigenic danger signals. In contrast, peptides presented by HLA class I molecules are derived from cytoplasmic proteins, that is, proteins generated within cells (Blum et al., 2013; Neeffjes et al., 2011; Townsend et al., 1986). These proteins are degraded by the proteases of the cellular proteasome, translocated into the endoplasmic reticulum, and subjected to N-terminal trimming by ERAP1 and ERAP2. Peptides having the appropriate anchor amino acids and size of usually 8 to 10 amino acids bind to HLA class I molecules. The complex of HLA molecule and peptide antigen is then expressed on the cell membrane for cognate antigen recognition by the T-cell receptors (TCRs) of CD8+ T cells.

The primary purpose of HLA class I molecules that are expressed on all nucleated cells is presentation of antigens from viruses that replicate intracellularly within infected cells and otherwise would escape immune recognition (Rock et al., 2016). The origin of HLA class I-presented peptide antigens from cytoplasmic proteins has essential consequences for understanding HLA class I-restricted pathogenic T-cell responses: an HLA class I-restricted autoimmune response must be directed against a particular target cell because it recognizes an antigenic peptide epitope of a cellular protein expressed by that cell (Neeffjes et al., 2011; Rock et al., 2016; Vyas et al., 2008).

**CD8+ T cells hold a key role in psoriasis**

According to this fundamental immunological principle, the HLA-C*06:02 association with psoriasis should reflect a clear-cut role and predispose for a CD8+ T-cell-mediated autoimmune response against a particular target cell type, and indeed, CD8+ T cells hold a pivotal position in psoriasis. Epidermal infiltration, activation, and clonal expansion of CD8+ T cells represent key events initiating psoriasis lesions (Conrad et al., 2007; Di Meglio et al., 2016), indicating an immune response against cellular antigens of a local target cell within the epidermis (Chang et al., 1994; Kim et al., 2012). Consequently, the HLA-C*06:02 association with psoriasis and the clonal CD8+ T-cell infiltrate in psoriasis lesions raised two directly related essential issues: defining the role of HLA-C*06:02 and identifying the target cells and autoantigens of the lesional psoriatic CD8+ T-cell response are crucial for elucidating the pathogenesis of psoriasis.

**UNBIASED ANALYSIS OF TCR SPECIFICITY**

**Generation of TCR hybridomas**

The TCRs of the pathogenic CD8+ T cells hold the clue to addressing these issues. They determine both HLA restriction and T-cell specificity. Analyzing this reactivity currently relies on recombinant TCRs because in human diseases the availability of pathogenic T cells is limited (Newell and Davis, 2014). This is the approach we have taken in psoriasis: we isolated epidermal CD8+ T cells from psoriatic skin lesions of HLA-C*06:02+ patients and determined the gene rearrangements of their paired TCR-α and β chains by a newly developed method (Kim et al., 2012). TCR-α/β genes of clonal CD8+ T cells, which actually designate the T cells involved in pathological circumstances (Newell and Davis, 2014; Woodsworth et al., 2013), were reconstituted in the TCR-deficient mouse T hybridoma cell line 58b-1. Along with the transfection of human CD8-β and NFAT-sGFP, the TCR hybridomas report on TCR signaling by robust expression of superfolder green fluorescent protein, which can be assessed by UV-fluorescence microscopy of FACS analysis (Seitz et al., 2006; Siewert et al., 2012). Because the TCR hybridomas carry the specificity of the lesional psoriatic CD8+ T cells, they can be used for defining HLA restriction, target cells, and autoantigens of the psoriatic T-cell response. In our experiments we focused on a particular Vα3S1/Vβ13S1 TCR because rearranging the Vβ13S1 TCR gene is paradigmatic for the infiltrating psoriatic CD8+ T cells (Chang et al., 1994), and the corresponding CD8+ T-cell clone was pervasive and was identified in two independent biopsy samples, indicating a broad relevance for the psoriatic immune response in that patient (Kim et al., 2012).

**Analyzing the specificity of the psoriatic T-cell response**

Through extensive co-culture experiments of the Vα3S1/Vβ13S1 TCR hybridoma with various HLA-C*06:02+ patients or -negative cell types from skin and other tissues, we observed that the Vα3S1/Vβ13S1 TCR reacts selectively against HLA-C*06:02+ or HLA-C*06:02− transfected primary melanocytes or melanocytic cell lines, irrespective of whether these cells originated from psoriasis patients or healthy individuals. The in vivo reactivity of the Vα3S1/Vβ13S1 TCR was reflected by the in situ distribution of CD8+ T cells in lesional psoriatic epidermis: immunohistological analysis of lesional biopsy samples from over 20 psoriasis patients showed that approximately 40% of CD8+ T cells directly contacted melanocytes. Extrapolating the contact frequency from the two-dimensional histological cutting planes into three dimensionality pointed to an even higher incidence (Fujisawa et al., 2015; Khimchenko et al., 2016; Wang et al., 2015). Polarization of granzyme B toward the contact sites with melanocytes clearly indicated that the T cells become activated through antigen recognition at the immunological synapse (Lieberman, 2003). Lytic granules constitute an important effector mechanism of CD8+ T cells that may occur without killing the target cell (Knittelbein et al., 2008). Indeed, staining of lesional melanocytes for cleaved caspase-3, a marker of apoptosis, could not detect signs of melanocyte death (Arakawa et al., 2015). Instead, numbers of melanocytes in psoriatic skin lesions were increased (Wang et al., 2013), presumably because the psoriasis signature cytokines IL-17A and tumor necrosis factor-α stimulate melanocyte proliferation. Simultaneously, they repress genes for melanogenesis and melanosome transfer, which may explain the hypopigmentation of psoriatic lesions,
which may turn into hyperpigmentation once the suppressive effects of IL-17A and tumor necrosis factor-α in resolving lesions have disappeared while melanocyte density is still increased (Wang et al., 2013). Thus, the unbiased analysis of the Vα3S1/Vβ13S1 TCR showed that melanocytes are the target cells of a non-cytotoxic lesional psoriatic CD8+ T-cell response, which is preferentially mediated by HLA-C*06:02 (Arakawa et al., 2015). This finding explains the skin-specific nature of psoriatic inflammation.

Proving melanocytes as targets of the lesional psoriatic T-cell response was vital for the identification of ADAMTSL5 as an HLA-C*06:02-presented autoantigen. TCRs are specific. They can react against a broad spectrum of antigenic peptides that share certain TCR-specific amino acid motifs defined by the HLA anchor residues and one or two contact amino acids at certain peptide positions. Amino acid positions outside this motif show greater tolerance for substitutions (Birnbaum et al., 2014). As a consequence, a single autoimmune TCR can recognize more than 10^6 different peptide ligands (Birnbaum et al., 2014; Mason, 1998; Sewell, 2012; Wooldridge et al., 2012). By extensive testing using plasmid-encoded combinatorial peptide libraries, we characterized the conserved amino acid motif of peptides ligating the Vα3S1/Vβ13S1 TCR in the context of HLA-C*06:02. We then searched the melanocyte transcriptome and human proteome for proteins containing peptide sequences corresponding to this amino acid pattern and finally identified six peptides from different natural human proteins that activated the Vα3S1/Vβ13S1 TCR when presented by HLA-C*06:02. Under natural conditions, however, cytoplasmic proteins are antigenic only if antigen processing and N-terminal trimming can generate the antigenic peptides from the full-length parent protein for HLA binding and TCR recognition (Blum et al., 2013; Strehl et al., 2005). When we tested the antigenicity of the full-length parent proteins of the six candidate peptide antigens, only ADAMTSL5 remained antigenic for the Vα3S1/Vβ13S1 TCR, and this antigenicity depended on expression of the ADAMTSL5 parent protein in melanocytes as psoriatic target cells. Thus, only the antigenic peptide from ADAMTSL5 could be generated by antigen processing from the parent protein, and this occurred only when ADAMTSL5 was expressed in melanocytes but not other cell types. Together with mutation analysis and mRNA knockdown, this proved that ADAMTSL5 is an HLA-C*06:02-restricted melanocyte autoantigen.

**ANTIGEN PROCESSING DETERMINES AUTOANTIGENS**

The actual immunogenicity of cellular proteins is determined by preferred proteasomal cleavage sites within the parent protein (Niedermann et al., 1995) and is influenced by cell-type specific differences in antigen processing. This limits the antigenic relevance of self-peptides to select proteins and tissues or cell types (Blum et al., 2013; Kniepert and Groettrup, 2014; Strehl et al., 2005). Accordingly, confirming a role as autoantigen requires that the antigenicity of HLA class I–presented candidate self-peptides has to be validated in the context of the full-length protein expressed within the target cell. Therefore, expression of ADAMTSL5 in the extracellular matrix of the skin does not argue against the role of ADAMTSL5 as psoriatic autoantigen, as recently suggested (Bonifacio et al., 2016). The intracellular expression and processing capabilities of melanocytes determine the role of ADAMTSL5 as an HLA class I-restricted melanocytic autoantigen, not the expression pattern in tissues.

These recent insights into the mechanisms of generating autoantigenic peptides from natural cellular proteins may also affect the relevance of potential psoriatic autoantigens reported in former studies. Some of them were identified by sequence homologies with proteins from Streptococcus pyogenes (Besgen et al., 2010; Gudmundsdottir et al., 1999) based on the hypothesis that streptococcal infection, a major psoriasis trigger, induces cross-reactive autoimmune responses against keratinocytes (Valdimarsson et al., 2009). Another potential autoantigen is LL-37 (Lande et al., 2014), a pleiotropic, multifunctional, 37-amino acid molecule generated by extracellular cleavage of the C-terminal part of the 170-amino acid cathelicidin antimicrobial peptide (Kahlenberg and Kaplan, 2013). Verification of the potential autoantigenic character for all these potential autoantigens was based on assays using peptides chosen according to HLA-C*06:02 anchor motifs. TCR binding degeneracy and size of the human TCR repertoire, however, would predict that peptides designed this way will likely induce T-cell activation irrespective of pathogenic relevance (Sewell, 2012). Without confirming that an HLA class I-presented peptide can be generated from the parent protein by antigen processing and presentation pathways within the target cell, a role as autoantigen for CD8+ T cells should therefore be interpreted with care.

**THE PATHOGENIC CD8+ T CELLS IN PSORIASIS: T-CELL RECRUITMENT OR SKIN RESIDENCY?**

An essential issue relates to the question of whether the pathogenic CD8+ T cells are skin resident memory T cells. Skin grafts of nonlesional skin from psoriasis patients transplanted onto immunodeficient mice developed into active psoriatic skin lesions (Boymann et al., 2004). Blockade of CD8+ T-cell migration into the epidermis, however, completely prevented formation of psoriatic hyperplasia in these experiments (Conrad et al., 2007). Accordingly, normal-appearing skin grafts contained pathogenic CD8+ T cells but still required T-cell recruitment into the epidermis for psoriasis metastasis. Therapeutic inhibition of T-cell emigration from lymph nodes by the S1P1 agonist with ponesimod and of T-cell trafficking from the circulation into psoriatic skin by the LFA-1 antibody with efalizumab improve psoriasis (D’Ambrosio et al., 2016; Jullien et al., 2004; Vaclavkova et al., 2014). In streptococcal-driven psoriasis, the lesional psoriatic T-cell clones were present in the cutaneous lymphocyte-associated antigen—positive fraction of tonsillar T cells, and tonsillectomy induced sustained psoriasis recovery (Diluvio et al., 2006; Thorleifsdottir et al., 2012). Together, these clinical observations indicate that ongoing psoriasis rapidly exhausts the pool of skin-resident pathogenic T cells and requires constant T-cell repopulation from lymphoid organs, where the same clones may persist as central memory T cells (Gaide et al., 2015).

**MELANOCYTES: TARGET CELLS IN SEVERAL AUTOIMMUNE SKIN DISEASES**

The autoimmune response in psoriasis shows that melanocytes are particularly immunogenic: Two other major autoimmune skin diseases, alopecia areata and vitiligo, involve...
melanocyte-specific CD8⁺ T-cell responses. Like psoriasis, they show strong family clustering and a complex genetic predisposition (Jin et al., 2016; Petukhova et al., 2010), but the genetic overlap with psoriasis is fairly limited (Elder, 2013). Disease outcome likely depends on T-cell differentiation: while in position (Jin et al., 2016; Petukhova et al., 2010), but the genetic inflammation (Lowes et al., 2008; Perera et al., 2012), melanocyte-specific CD8⁺ T cells, which have been conditioned during thymic T-cell development (c) under the influence of gene variants affecting CD8⁺ T-cell differentiation. (d) Gene variants related to IFN-γ signaling and NF-κB activation induce an increased inflammatory response which (e) provides the secondary signals for (f) autoreactive T-cell activation. (g) Gene variants related to the IL-23/IL-17A axis maintain activation of (h) CD8⁺ Tc17 cells, which promote psoriatic inflammation through the psoriasis signature cytokines IL-17A, IL-17F, IFN-γ, and TNF-α. Tc17, IL-17-producing CD8⁺ T-cell; TCR, T-cell receptor; TNF-γ, tumor necrosis factor-γ.

**CONCLUSION**

Overall, psoriasis represents a paradigmatic human immune-mediated disease, where the unbiased, that is, hypothesis-free, analysis of a pathogenic TCR from the lesional T-cell infiltrate could now clarify the role of the main HLA risk allele, identify the target cells and an autoantigen, and thus establish the autoimmune nature of the pathogenic immune response. This represents an unequivocal proof of the pathogenic role of a disease-associated HLA allele because it is based on ex vivo human material and not on experimental animal models. As a still unique TCR-based approach of its kind, which has been conducted successfully in medical research for elucidating the pathogenesis of an immune-mediated human disorder, the use of the pathogenic TCR in psoriasis now definitely proves that psoriasis is an autoimmune disease. It depends on an HLA class I-restricted CD8⁺ T-cell mediated autoimmune response against melanocytes. The main psoriasis risk allele, HLA-C*06:02, apparently confers susceptibility to psoriasis by promoting this melanocyte-specific autoimmunity through autoantigen presentation. Together with the identification of ADAMTS5 as a melanocyte autoantigen, these results now allow for redefining the cascade of pathogenic events in psoriasis, defining an HLA class I-restricted autoimmune response against melanocytes as the central pathogenetic event.

Autoimmune diseases are thought to arise from a combination of genetic and environmental factors. According to current concepts, polymorphisms in various genes may result in defective control and reduced thresholds for activation of innate and specific immunity, which may overcome the horror autotoxicus and promote autoimmunity when environmental factors activate self-reactive T cells (Rosenblum et al., 2015; Steinman, 1995). As “iatrogenic” support for this concept, checkpoint inhibition for treating melanoma metastasis induced new psoriasis onset in patients with high expression of ADAMTS5 in the melanoma cells (Nonomura et al., 2017). Under “regular” conditions, initiation and maintenance of the melanocyte-specific autoimmune response likely occurs only when additive effects of gene variants related to increased type I IFN signaling (ELMO1, TYK2, SOCS1, IFIH1, RNFI14, IRF4, RIG1/DDX58, IFNLR1/IL28RA, IFNGR2, TNFAP3, TNIP1, TYK2, REL, NfκBIA, CARD14, CARD11, UBE2L3, FBX19, etc.) and TNF-α signaling: IL-17A, IL-17F, IL-22, IFN-γ, etc. (b) Thymic CD8⁺ T-cell maturation; (c) Gene variants related to thymic CD8⁺ T-cell maturation: ETS1, RUNX3, TNFRSF9, MBD2, IF4, etc. (d) Gene variants related to IFN & NF-κB pathways: ELMO1, TYK2, SOCS1, IFIH1, RNFI14, IRF4, RIG1/DDX58, IFNLR1/IL28RA, IFNGR2, TNFAP3, TNIP1, TYK2, REL, NfκBIA, CARD14, CARD11, UBE2L3, FBX19, etc.; (e) Gene variants related to antigen presentation HLA-C*06:02, ERAP1, etc. (f) Autoreactive T-cell activation.
FBXL19), N-terminal antigen trimming (ERAP1), thymic CD8+ T-cell differentiation (ETS1, RUNX3, TNRFSF9, MBDB2, IRF4), and the IL-23/IL-17A axis (IL23R, IL12B, IL12RB, IL23A, IL23R, TYK2, STAT3, STAT5A/B, SOCS1, ETS1, TRAF3IP2, KL4, IF3) provide sufficient co-stimulatory proinflammatory signals, generate the appropriate peptide epitope, enhance IL-23 and IL-17A signaling, and maintain IL-17–secretting CD8+ T-cell differentiation (Figure 1). In accordance with this view, HLA-C*06:02 associated equally strongly with mild and severe psoriasis in a case-case study. Strong additive effects for severe disease states were observed when HLA-C*06:02 combined with gene variants of IL23A, IL23R, IL12B, NFKB1, or TNIP1 (Nikamo et al., 2015). Thus, HLA-C*06:02 confers an overall risk for psoriasis by facilitating an autoimmune response against melanocytes through autoantigen presentation, and gene variants related to innate immune activation and the IL-23/IL-17A axis may modify disease expression.

CONFLICT OF INTEREST
The author states no conflict of interest.

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