

Autoantigens act as tissue-specific chemoattractants

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Abstract: We have investigated the chemoattractant properties of self-antigens associated with autoimmune diseases and solid tumors. Many autoantigens induced leukocyte migration, especially by immature dendritic cells (iDC) by interacting with various chemoattractant Gi-protein-coupled receptors (GiPCR). Our initial observation that myositis-associated autoantigens, histidyl-tRNA synthetase and asparaginyl-tRNA synthetase, were chemotactic for CC chemokine receptor 5 (CCR5)- and CCR3-expressing leukocytes, while other nonautoantigenic aminoacyl-tRNA synthetases were not, suggested that only self-antigens capable of interacting with receptors on antigen-presenting cells were immunogenic. We next determined that self-antigens associated with autoimmune diseases, e.g., multiple sclerosis or experimental autoimmune encephalomyelitis, type I diabetes, scleroderma, systemic lupus erythematosus, autoimmune uveitis, or experimental autoimmune uveitis (EAU), were chemotactic for GiPCR expressed by iDC. The majority of autoantigens were DC chemoattractants at 10–100 ng/ml, but did not induce DC maturation until they reached 1000-fold higher concentrations. Interphotoreceptor retinoid-binding protein and retinal arrestin (S-antigen) are targets of autoantibodies in human uveitis and are chemotactic for CXC chemokine receptor 5 (CXCR5)- and/or CXCR3-expressing iDC. However, although S-antigen does not induce EAU in wild-type mice, it is nevertheless a chemoattractant for murine iDC. These unexpected observations suggested that the chemotactic activity of these tissue-specific self-antigens could be involved in promotion of tissue repair and restoration. Thus, the primary role of autoantigens may be to alert the immune system to danger signals from invaded and damaged tissues to facilitate repair, and autoimmune responses subsequently develop only in subjects with impaired immunoregulatory function. *J. Leukoc. Biol.* 77: 854–861; 2005.

Key Words: chemokines · tumor antigens · chemotaxis · migrations · dendritic cells

INTRODUCTION

Autoimmune diseases arise as a consequence of the breakdown in tolerance to self-antigens, and the diagnosis may be confirmed based on the detection of characteristic autoantibodies [1]. In some autoimmune diseases, autoantibodies and autoimmune T cell responses develop against unique antigens typical of the involved tissues and organs, e.g., insulin in type I diabetes, interphotoreceptor retinoid-binding protein (IRBP) in autoimmune uveitis, and myelin basic protein (MBP) or proteolipid protein (PLP) in multiple sclerosis (MS). In contrast, some autoimmune patients develop autoantibodies directed against proteins and nucleotides present in every nucleated cell in the body, such as single-stranded DNA (ssDNA) in systemic lupus erythematosus (SLE), histidyl-tRNA synthetase (HisRS) in myositis, and topoisomerase I in scleroderma. Nevertheless, the repertoire of the targets of autoantibodies is surprisingly restricted, and often, they are characteristic of only one autoimmune state. These observations raised several questions: Why are these particular tissue antigens, which represent probably less than 1000 of our 30,000 antigens, more likely to break tolerance to self? As serum autoantibodies and autoimmune T cell responses frequently appear only with disease progression, do they play a passive or active role in disease initiation? Why are certain tissues characterized by the restricted development of autoantibodies and autoimmune T cell responses to widely dispersed autoantigens? Many immunologists have confronted these issues, and this brief review will certainly not resolve them, but our observations that many autoantigens are chemotactic for mononuclear leukocytes may provide some clues that lead to new perspectives. The chemotactic capacity of autoantigens not only can result in their attracting immune cell subsets to a given tissue but also indicates that they are interacting with cell membrane receptors. We propose that this receptor interaction also promotes

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the internalization, processing, and presentation of the autoantigens, thus enhancing their immunogenic capabilities.

Over 40 chemokines have been identified as major traffic directors of leukocytes engaged in innate and adaptive immune responses [2]. The subset of leukocytes responding to particular chemokines is based on their interaction with selected G α protein-coupled receptors (GiPCR) [3]. This interaction can be blocked by pertussis toxin (PTX), which is known to inhibit G α iPCR-mediated signal transduction [4]. It was therefore surprising to learn that proteolytic cleavage of tyrosyl-tRNA synthetase (TyrRS) yielded an NH₂-terminal domain, composed of amino acids 1–364, which was chemotactic for neutrophils [5]. The chemotactic effect of TyrRS was inhibited by PTX. TyrRS interacts with a CXC chemokine receptor (CXCR1) on human neutrophils, despite the fact that it has no sequence homology with the chemokine cysteine motif of interleukin-8 (IL-8; CXC chemokine ligand 8), a known ligand for CXCR1 [5]. This observation suggested that other aminoacyl-tRNA synthetases (aaRS) might possess chemotactic activity.

AUTOANTIGENS ARE CHEMOTACTIC FOR MONONUCLEAR CELLS

We therefore investigated the possibility that other aaRS might be chemotactic for leukocytes. Unlike TyrRS, HisRS and asparaginyl-tRNA synthetase (AsnRS) are known to be targets of autoantibodies that are detected in up to 25% of patients with autoimmune myositis. These aaRS did not induce intracellular calcium flux but do activate extracellular signal-regulated kinase-2, and they had chemotactic effects that were inhibited by PTX. We showed that intact HisRS and a 1–48 amino acid peptide were chemotactic for human CD4⁺ and CD8⁺ lymphocytes, and IL-2 activated monocytes that express CC chemokine receptor 5 (CCR5) but not for neutrophils or unstimulated monocytes that do not express CCR5 [6]. AsnRS similarly was chemotactic for CCR3-expressing mononuclear cells [6]. Although, these aaRS stimulated cells to migrate only two- to fourfold over background and were therefore not as efficacious as chemokines, they were potent, requiring only low (subnanomolar) concentrations to induce migration in *in vitro* Boyden chamber assays. Their chemotactic activity was also readily inhibited (desensitized) by prior exposure of cells to the appropriate chemokine ligands, and conversely, HisRS partially desensitized the chemotactic response to regulated on activation, normal T expressed and secreted [RANTES; CC chemokine ligand 5 (CCL5)].

The structural relationship between HisRS and CCL5 was studied. There is no amino acid homology among HisRS, AsnRS, and chemokines. The NH₂ terminal (1–48 HisRS) domain of HisRS, containing the principal antigenic site of HisRS, was chemotactic for mononuclear cells, and a deletion mutant lacking this domain (M-HisRS) was not. Neutralizing antibodies directed against the second extracellular loop (third extracellular domain) of CCR5 blocked HisRS as well as CCL4- and CCL5-induced chemotaxis. Conversely, CCL5 and HisRS interfered with the binding of this antibody to CCR5. The use of chimeric receptors consisting of various portions of

CCR5 and CCR2 (kindly provided by Dr. Israel Charo, University of California, San Francisco) did differentiate the chemotactic responses of HisRS and CCL5 and suggested that HisRS interacts predominantly with the third and to a lesser extent, with the fourth extracellular domain of CCR5, and CCL5 interacts with the first as well as the third. Thus, HisRS may be interacting with receptor domains distinct from those used by the chemokines.

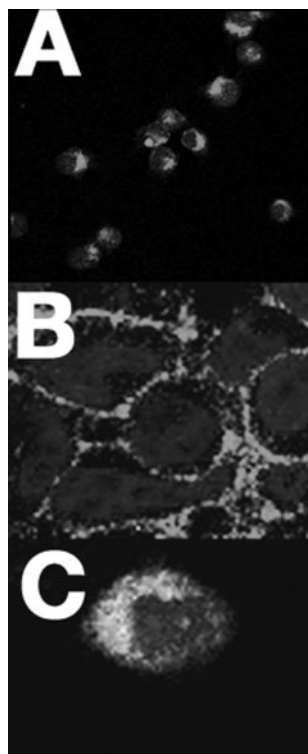
AUTOANTIGENS ARE CHEMOTACTIC FOR IMMATURE DENDRITIC CELLS (iDC)

As antigen-presenting DC activate adaptive immune responses, the capacity of autoantigens to interact with DC is requisite for the induction of T-dependent immune responses. It is known that iDC express a wide variety of chemokine receptors, which are lost as they mature into mature DC (mDC; as reviewed in ref. [7]). Indeed, HisRS and AsnRS were chemotactic for iDC, which express CCR5 and CCR3, but not mDC, which do not. Furthermore, a number of nonautoantigenic synthetases, such as aspartyl and lysyl-tRNA synthetases, were not chemotactic for mononuclear cells including iDC. This led us to suggest that chemotactic autoantigens may, at a minimum, be involved in recruiting mononuclear antigen-presenting cells (APC) to sites of tissue damage and inflammation. In addition, our recent collaborative studies have shown that HisRS, just like chemokines, is internalized only by CCR5-expressing cells, whereas cells that do not express CCR5 do not bind fluorescent-tagged HisRS (**Fig. 1** and unpublished data). Further, HisRS colocalizes in the endocytic compartment with major histocompatibility complex (MHC) class II antigen (**Fig. 2** and unpublished observations). This suggests that interaction of autoantigens with GiPCR results in their localization in antigen-processing compartments. Furthermore, the possibility that interaction of these autoantigens with GiPCR may promote their uptake, processing, and presentation by iDC has also been suggested by studies showing that antigen-receptor interactions, including those with GiPCR, markedly increase the immunogenicity of self and nonself antigens [8–11]. Thus, autoantigens interact with receptors and appear to be more than just passive targets of autoantibodies/autoreactive T cells in autoimmune disease and potentially, can be actively involved in breaking tolerance to self. We therefore proceeded to test a number of autoantigens from a variety of autoimmune states for their chemotactic effects on iDC.

MANY AUTOANTIGENS ARE CHEMOTACTIC

We have established that most self-proteins that are not involved in autoimmune responses are not chemotactic (**Table 1**). For example, a number of serum proteins, HSPs, and aaRS, as discussed, are not chemotactic. Although it could be argued that lack of a chemotactic response to highly abundant proteins, such as IgG and albumin, might be a result of constant desensitization of the receptors, this is unlikely to be the case for intracellular proteins such as ufd-2 and the lysyl and tryptophanyl-tRNA synthetases. Furthermore, the classic che-

Fig. 1. AlexaFluor-tagged HisRS and RANTES induce capping of CCR5/human embryo kidney (HEK) cells. (A) Human CCR5-expressing HEK cells (CCR5/HEK) were exposed to Alexafluor®-488, tagged for 20 min at 37°C, and then visualized with a confocal microscope. There appears to be increased fluorescence in one plane, suggestive of ligand-induced polarization or capping. HEK cells not expressing CCR5 do not bind HisRS and can therefore not be visualized. (B) CCR5/HEK cells were stained at time 0 with fluorescein isothiocyanate (FITC)-anti-CCR5 (green) and 4',6-diamidino-2-phenylindole (DAPI; blue) for nuclear stain and then were visualized by confocal. (C) CCR5/HEK cells were exposed to untagged, 1 µg/ml RANTES (CCL5) for 20 min at 37°C and then were stained with FITC-anti-CCR5 (green), DAPI (blue), and T3166, a rhodamine-conjugate membrane dye (red), followed by visualization. As in A, there appears to be ligand-induced, receptor capping that precedes internalization.

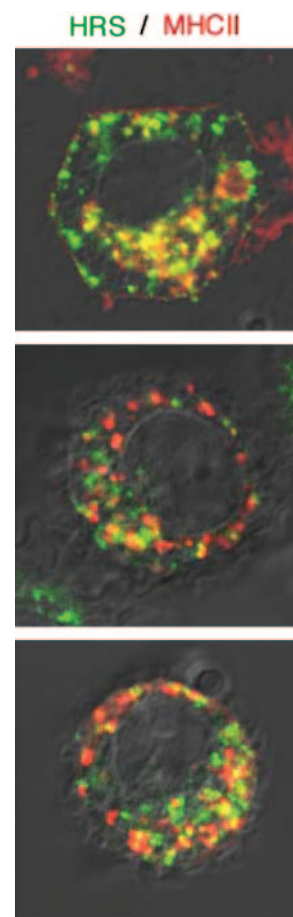


moattractant proteins, chemokines and anaphylatoxins, induce cell migration without inducing autoantibodies or autoreactive T cells unless administered with an adjuvant or structurally modified by fusion to an antigen [12–15]. Conversely, many self-proteins targeted by autoantibodies or autoreactive T cells induce GiPCR-dependent chemotaxis (**Table 2**).

We tested two intact, functional proteins associated with MS: PLP (provided by Drs. Marjorie Lees and O. Koul, Shriver Center, University of Massachusetts Medical School, Waltham) and MBP. Both were chemotactic for human iDC. Furthermore, although MBP was only chemotactic at micromolar (10,000 ng/ml) concentrations, PLP was most effective at lower concentrations (100 ng/ml; **Fig. 3**). We further investigated several peptide domains from PLP, MBP, and MOG, which are proteins capable of inducing EAE, and observed that some fragments that were known to be capable of inducing EAE were chemotactic (**Table 3**). Two nucleolar proteins, fibrillarin (U3-RNP) and topoisomerase I [provided by Dr. Paul Hu, National Cancer Institute (NCI), Frederick MD], which are autoantigens associated with scleroderma, were chemoattractants for human monocytes (data not shown). Similarly, a number of autoantigens associated with type 1 diabetes, especially the phosphatase IA-2 (provided by Dr. Abner Notkins, National Institute of Dental and Craniofacial Research, Bethesda, MD) and transaldolase (provided by Dr. Andras Perl, State University of New York at Syracuse), were chemotactic for iDC (**Fig. 3**). Additionally, the retinal antigens, S-antigen and IRBP, which induce experimental autoimmune uveitis (EAU), as well as several peptides derived from them, were chemotactic for murine and human iDC (**Fig. 3** and ref. [16]).

In contrast, some autoantigens, such as periaplain, vimentin, SRP54, -68, and -72, B32, ssDNA, and La, failed to induce leukocyte migration at tested concentrations. We have not

Fig. 2. HisRS (HRS) is internalized to endocytic vesicles, including MHC II-containing compartments. Human iDC were exposed to Alexafluor®-488-tagged HisRS (1 µg/mL) for 20 min at 37°C. The cells were subsequently fixed, permeabilized, and stained with anti-MHC II (L243, ATCC), detected with Texas red-labeled secondary antibodies, and the images were visualized by confocal microscopy.



tested these antigens over a wider concentration range as yet, and like MBP and transaldolase, some of them may be chemotactic only at micromolar concentrations. The failure of La and ssDNA to be chemotactic was rather disappointing. The possibility they might be active as complexes was therefore investigated. This revealed that mixtures of femtomolar concentrations of ssDNA and nanomolar concentrations of La were chemotactic for iDC (**Fig. 4**), and this response was also inhibited by PTX, implicating an interaction of the complex

TABLE 1. Proteins and Autoantigens Not Chemoattractant for iDC

Proteins	Class
HSP-60 and -90	Self-proteins
Thrombin	
Human serum IgM and IgG	Autoantigens
Human serum albumin (chemokinetic)	
Ubiquitination fusion degradation protein-2 (ufd-2)	
Lysyl- and tryptophanyl-tRNA synthetases	
Select chemokines and anaphylatoxins	
SRP 68 and 72 (myositis)	
Periaplain (rod and tail; pemphigus)	
Vimentin (SLE and Sjogren's)	
La-**	
ssDNA**	
NY-ESO-1 (melanoma)	Tumor antigens
Prostate-specific antigen (PSA)	

** Alone. HSP, Heat shock protein; IgM and IgG, immunoglobulin M and G, respectively; SRP, signal recognition particle; La, lupus anticoagulant.

TABLE 2. Autoantigens Chemotactic for iDC

Protein	Source of tested protein or peptide	Disease
Histidyl-tRNA synthetase (HRS)	RB, RE, S	Myositis
Asparaginyl-tRNA synthetase (NRS)	RE	
Aspartyl-tRNA synthetase (DRS)	RE	Scleroderma
Fibrillarin (U3-RNP)	RE	
Topoisomerase 1	RB	
Interphotoreceptor retinoid-binding protein (IRBP)	N, RE, S	Uveitis
Retinal arrestin (S antigen)	N, RE, S	
Myelin basic protein	N, RE	EAE/MS
MOG and peptide domains	S	
PLP and peptide domains	N, S	
Transaldolase	R	Type 1 diabetes
IA-2	RE	
Transaldolase	R	
Gp100	RE	Neoplasias
MUC 1	N, RE, S	
CEA	RE	SLE
ssDNA plus La		

R, Recombinant; RB, recombinant in baculovirus; RE, recombinant in *Escherichia coli*; S, synthetic; N, natural source; U3-RNP, U3 nucleolar ribonucleoprotein; MOG, myelin oligodendrocyte glycoprotein; EAE, experimental autoimmune encephalomyelitis; IA-2, islet antigen 2; MUC 1, mucin 1; CEA, carcinoembryonic antigen.

with GiPCR. Based on reports that immunization of mice with tumor antigens fused to ligands for GiPCR results in enhanced immune responses to weak tumor antigens [15], we postulated that those self-antigens interacting with GiPCR are more capable of inducing autoimmune responses. It is also entirely possible that some autoantigens use nonchemotactic receptors such as scavenger receptors to enter the antigen-processing endocytic pathway [8, 10]. Overall, our data support the idea that the majority of self-antigens that are involved in autoimmune reactions may be those that are preferentially taken up by receptors targeting antigen-processing pathways in APC.

TUMOR AUTOANTIGENS ARE CHEMOTACTIC

It has been clearly documented that cancer patients often develop autoantibodies to their tumor antigens [17]. Furthermore, certain melanoma patients immunized with melanocyte or melanoma-derived antigen vaccines developed autoimmune vitiligo [18]. We therefore investigated the chemotactic properties of some tumor antigens. We tested Good Manufacturing Practice preparations of gp100 and NY-ESO-1 (provided by Drs. Steven Rosenberg and Paul Robbins, NCI) and to our surprise, consistently obtained a chemotactic response of iDC, monocytes, and T cells to nanomolar concentrations of gp100 (Fig. 3) but not at all to NY-ESO-1. Perhaps this difference is a result of the fact that NY-ESO-1 is a fetal and testis antigen and is therefore not systemically expressed after birth. Based on desensitization of the chemotactic response to gp100 by monocyte chemoattractant protein-1/CCL2 and the selective capacity of CCR2-transfected HEK293 cells to migrate in response to gp100, we concluded that gp100 interacts with CCR2. Furthermore, gp100 interacts with human monocytes, T lymphocytes, and iDC, which are known to express CCR2. In addition, human gp100 is more chemotactic for normal murine iDC than for iDC from CCR2^{-/-} mice (unpublished obser-

vations). Thus, gp100 behaves like an autoantigen with respect to chemotaxis and the participation of gp100 peptides in vaccine-induced autoimmune vitiligo in some of the patients experiencing tumor regression [19].

In collaboration with C. Carlos and O. Finn (University of Pittsburgh Medical School, PA) we have studied a more typical tumor antigen, MUC 1 [20]. The normal counterpart of MUC 1 is heavily glycosylated and expressed on the surface of normal gastrointestinal epithelial cells, while MUC 1 derived from pancreatic and intestinal tumors is hypoglycosylated. Only the latter is chemotactic for a PTX-inhibitable GiPCR on iDC but not for mDC. Furthermore, we have also determined that CEA is chemotactic for iDC (data not shown), while PSA derived from prostate tumors is not (kindly provided by Dr. Jeffrey Schlom, NCI). Perhaps the detection of chemotactic activity can identify candidate tumor antigens for tumor vaccine therapy.

THE CHEMOTACTIC AND PATHOGENIC ACTIVITIES OF AUTOANTIGENS ARE DISSOCIABLE

We explored several EAU autoantigens, namely S-Ag arrestin and IRBP, in greater detail, as they are not only putative targets of autoantibodies/autoreactive T cells in human autoimmune uveitis but they are capable of inducing EAU in rodents [16]. Rodent, bovine, and human uveitic autoantigens are evolutionarily conserved and are interchangeable. We determined that S-Ag and IRBP are chemotactic for normal human iDCs and lymphocytes but not phagocytic cells. Cross-desensitization studies and evaluation of the chemotactic responses of cells transfected to express only one of the chemokine receptors revealed that IRBP interacts with CXCR5 and CXCR3, and the chemotactic response of S-Ag required only the CXCR3 chemokine receptor.

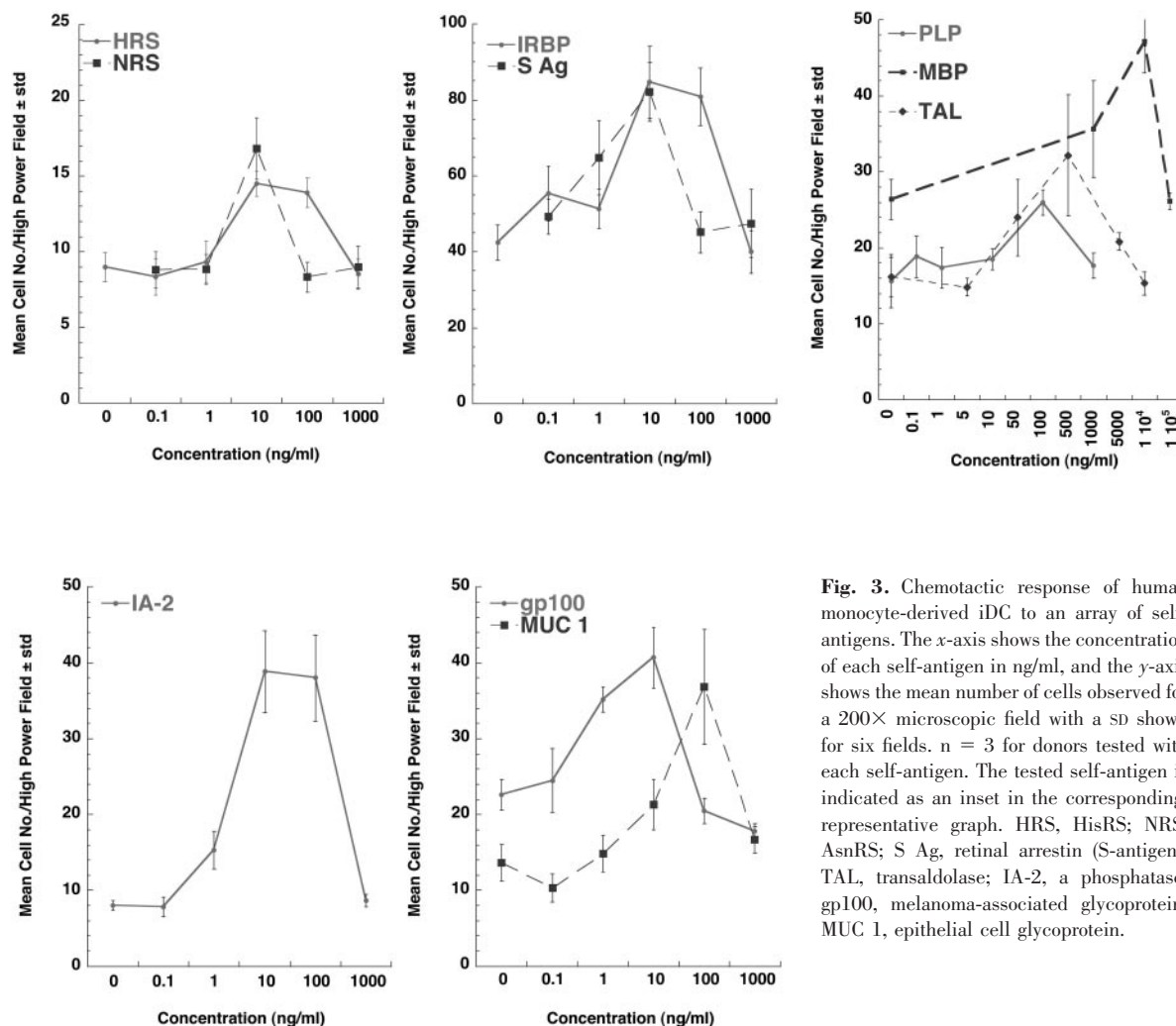


Fig. 3. Chemotactic response of human monocyte-derived iDC to an array of self-antigens. The x-axis shows the concentration of each self-antigen in ng/ml, and the y-axis shows the mean number of cells observed for a 200 $\times$ microscopic field with a SD shown for six fields. $n = 3$ for donors tested with each self-antigen. The tested self-antigen is indicated as an inset in the corresponding, representative graph. HRS, HisRS; NRS, AsnRS; S Ag, retinal arrestin (S-antigen); TAL, transaldolase; IA-2, a phosphatase; gp100, melanoma-associated glycoprotein; MUC 1, epithelial cell glycoprotein.

The availability of a good mouse model for EAU enabled us to compare resistant mouse strains with those sensitive to the induction of EAU. This revealed that iDC from resistant and sensitive mice exhibited equal chemotactic responses to both the autoantigens. Furthermore, unlike IRBP, S-Ag is not able to induce uveitis in wild-type mice [16, 21]. This suggested that the chemotactic effect of these autoantigens could be dissociated from their capacity to induce EAU.

This issue was investigated further by using 20 amino acid-overlapping, synthetic human peptide epitopes from IRBP with well-established uveitogenic (pathogenic) and antigenic (lymphoproliferative) capabilities. This revealed that some of the peptides were chemotactic, similar to the intact IRBP for normal human lymphocytes. However, some of the smaller IRBP peptides, which were reported to be uveitogenic in mice, were not chemotactic for human iDC. Conversely, the chemotactic activity of peptides could be dissociated from their ability to induce lymphocytes from patients with uveitis to proliferate. These data also suggested that the chemotactic effect of autoantigens is not necessarily related directly to the capacity to induce autoimmune processes. This was reinforced by studies of peptides associated with EAE. As shown in Table 3, several of the peptides that were not chemotactic for human monocyte-derived iDC did induce EAE in various animal

models. Additionally, most of the peptides that induced human PBMC to proliferate did not induce EAE. Taken together, our observations suggest that although recruitment of iDC by self-antigens may contribute to the development of immune tolerance or autoimmune disease, it is not the only mechanism.

AUTOANTIGENS FAIL TO INDUCE MATURATION OF DC

The fact that an autoantigen or tumor antigen is chemotactic does not necessarily enable it to also serve as an immunogenic stimulant. This is borne out by the observations that the gp100 melanoma tumor antigen, which is capable of interactions with a GiPCR, even when used in vaccines together with DC, induced little or no tumor immunity. For example, it was recently reported that gp100 administered alone was not immunogenic and was not capable of inducing T cell-mediated killing of melanoma in clinical studies [22]. Only in the presence of multiple proinflammatory signals that induce B7/CD40-costimulant molecules on DC can autoantigens overcome tolerogenic/suppressive signals and generate autoimmune reactions, which result in effective tumor immunity.

TABLE 3. Activity of MBP, PLP, and MOG Peptides

Peptide	Ave. chemotactic index* for human monocyte-derived iDC	Lymphoproliferative activity for human PBMC	Induction of EAE**
MBP 13–32	1.45	+++	–
MBP 83–99	2.0	++	++
MBP 111–129	1.9	+++	+
MBP 131–155	1.7	+	–
MBP 146–170	–	+++	+
PLP 89–106	–	+	–
PLP 133–154	3.1	+++	+++
PLP 178–197	–	+	+
PLP 190–209	–	+	–
MOG 35–55	–	+++	++
MOG 11–30	–	+	–
MOG 21–40	1.9	+	–
MOG 1–20	–	+++	–
CNPase 343–373	1.8	++	–
CNPase 356–388	1.8	++	–
Flu-HA 393	–	+++	–

* Chemotactic index, Ratio of migratory response to 100 ng/ml peptide to control; –, no migration. ** In mouse, rat, rhesus monkey, or guinea pig models. PBMC, Peripheral blood mononuclear cells; CNPase, 2',3'-cyclic nucleotide 3-phosphodiesterase; Flu-HA, Flu-hemagglutinin.

Thus, the process of autoimmunity and tumor immunity is closely related, but although autoimmunity results in undesirable and destructive cellular immune reactions to self-antigens, this is often not the case in cancer patients, who instead, unfortunately, develop no response or tolerogenic responses to their “self” tumor antigens.

As two signals, namely antigens and costimulants, are required to induce immune responses, we have carefully evalu-

ated the capacity of chemotactic auto and tumor antigens to induce the maturation of iDC into mDC. To be fully functional, mDC need to express costimulatory molecules such as MHC II antigens and CD83 or CD86. They also need to produce immunostimulating cytokines such as IL-12p70. Further, DC need to express CCR7 receptors to enable them to migrate to draining lymph nodes, where the processed antigen can be presented, resulting in an immune response.

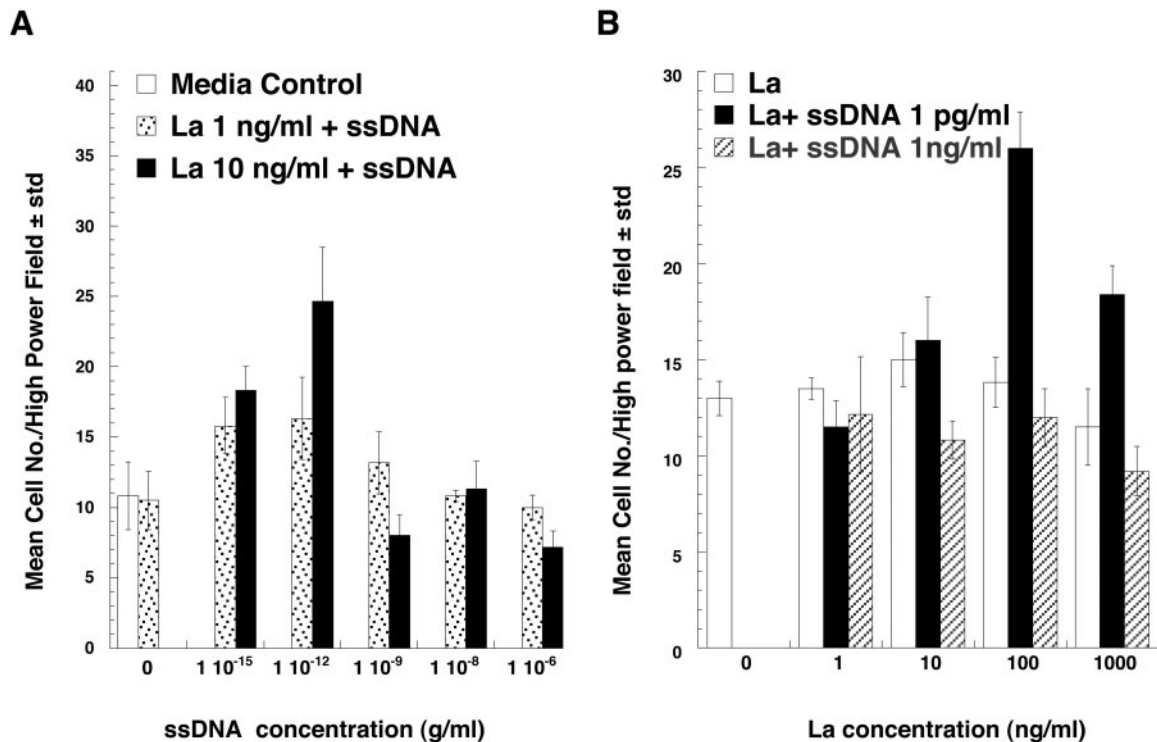


Fig. 4. The chemotactic effects of La and ssDNA on human monocyte-derived iDC. (A) Effect of addition of low concentrations of ssDNA (1×10^{-15} – 1×10^{-6} g/ml) to 1 or 10 ng/ml La on the chemotactic response of iDC. (B) Effect of addition of 1–1000 ng/ml concentrations of La to 1 pg or 1 ng/ml ssDNA on the chemotactic response of iDC.

Our experiments clearly showed that at subnanomolar concentrations, HisRS and gp100 were chemotactic, but they did not induce maturation of iDC. Only at 20-fold greater concentrations were these autoantigens able to induce low-to-moderate levels of expression of CD40, CD80, MHC II antigens, and CCR7 by iDC (unpublished observation). At the higher self-antigen concentrations, iDC were stimulated to produce low levels of some cytokines and chemokines, including IL-2, IL-12p40, and tumor necrosis factor (TNF), and somewhat higher levels of IL-6, IL-10, macrophage-derived chemokine/CCL22, and thymus and activation-regulated chemokine/CCL17. Consequently, these chemoattractants are able to induce iDC to mature only partially at "pharmacological" concentrations but not at their chemotactic concentrations.

CONCLUSIONS

What, therefore, is the relevance of the interaction between autoantigens and chemotactic receptors? As many of these autoantigens are intracellular moieties, they are not normally available to cell-surface receptors but may be released in the course of injurious insults resulting in cell death. We therefore propose the following scenario: Injury induces the release of self-antigen, resulting in a tissue-specific signal to cells of the host innate and adaptive immune system to migrate to the site of injury and eliminate pathogens and cellular debris, deliver growth factors and other mediators that will facilitate repair, and restore homeostasis. As Casciola-Rosen and colleagues [23] have shown, selected autoantigens may be preferentially up-regulated in inflamed tissues. As tissue injury is associated with the in situ production of proinflammatory cytokines such as IL-1, TNF, and chemokines, the cellular infiltration may be amplified, macrophages may be activated, and DC may be stimulated to mature. The mDC could deliver the preferentially processed autoantigenic peptides to T effector cells in draining lymph nodes, thus engaging adaptive immune responses. As the injury subsides, and cytokine production ceases, tissue-resident DC will remain immature and will interact with T regulatory cells to reestablish peripheral tolerance/anergy. There are reports in the literature that support such a scenario. Michal Schwartz and Kipnis [24] have shown that prior immunization of rats with the MBP autoantigen promotes the repair and recovery of rats from spinal cord and optic nerve injuries. They observe increased infiltration of the sites of injury by macrophages in rats immunized with MBP, resulting in more effective degradation and removal of tissue debris and facilitation of subsequent neural tissue repair. Although those rat strains genetically proven to develop EAE go on to do so, the resistant strains repair equally well without developing EAE. A parallel situation involving retinal ganglion cell neuroprotection is observed with respect to retinal antigens in mouse strains susceptible or resistant to EAU [25]. Thus, the development of autoimmune sequelae in response to the autoantigen is based on genetic or other deficiencies, independent of their role as harbingers of tissue damage and mobilizers of the process of cellular repair. Presumably, genetic or acquired immune defects can result in a failure to turn off the reparative immune response, resulting in progressive expansion of the

clone of self-reactive T cells and consequent progression to self-destructive autoimmune reactions.

Therefore, our current responses to the three questions posed in the introduction are as follows: Autoantigens, by interacting with receptors on leukocytes, may fulfill a primary role of alerting the innate and adaptive immune systems to danger signals from damaged tissues but induce autoimmune reactions only in the case of dysregulated, suppressive immune responses. Perhaps the reparative role in normal host defense is facilitated by the tissue specificity of many self-antigens. However, in patients with immunoregulatory defects, tissue damage as a result of injury or invasion by microorganisms may initiate immune responses that persist, despite repair of the damage, and culminates in inappropriate autoimmune, self-destructive reactions.

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