The HLA-B*5101 Molecule-Binding Capacity to Antigens Used in Animal Models of Behçet's Disease: A Bioinformatics Study

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ABSTRACT: **Background:** The human lymphocyte antigen (HLA) molecule B*5101 is a functioning receptor of the immune system and is generally accepted as a genetic marker for Behçet disease (BD), a multi-organ, chronic inflammatory disorder. The role of the HLA-B*5101 in the pathogenesis of BD is elusive. The assumption that HLA-B*5101 has an active role in BD is suggestive, but no antigen has yet been identified.

Objectives: To evaluate the potential binding capacity of various antigens to the HLA-B*5101 molecule.

Methods: Using bioinformatics programs, we studied the binding capacity of HLA-B*5101 and its corresponding rat molecule RT.A1 to the following antigens: heat shock protein-60 (HSP60), major histocompatibility complex class I chain-related gene A (MICA), retinal S-antigen (S-Ag), HLA-B27 molecule and its peptide (PD) and tropomyosin (TPM), all of which serve as antigens in animal models corresponding to BD.

Results: In each protein including the B*5101 molecule itself, the computerized programs revealed several short sequences with potential high binding capacity to HLA-B*5101 with the exception of B-27PD. The rat MHC RT1. Al. had no binding capacity to S-Ag.

Conclusions: The evaluated proteins have the potential to bind to and to serve as potential antigens to the HLA-B*5101 and the rat MHC RT1.Al. molecules. The pathogenicity of these suggested short peptides should be evaluated in animal models of BD.

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KEY WORDS: Behcet's disease (BD), autoantigens, animal models, HLA-B*5101

Behçet's disease is an auto-inflammatory, multi-organ vasculitis of unknown cause. Ethnic origin is one of the factors that modulate the manifestations and prevalence of BD; indeed, the disease is more prevalent in Asia, extending from the Mediterranean basin to Japan. Approximately 80% of patients with BD bear the human lymphocyte antigen-B51 molecule. However, it is not clear whether the HLA -B51 represents simply an association with BD or if the HLA-B51 molecule has a direct role in the pathogenesis of the disease [1].

Several animal models have been utilized to explore the etiopathology of BD. T cell receptor binding studies demonstrated that four epitopes derived from microbial heat shock protein 60/65 share homology with human HSP 60 [2]. Furthermore, immunization or oral administration with peptide 336-351 derived from HSP65-induced experimental uveitis without other symptoms of BD [3]. An immunogenetic explanation for the involvement of HSP in BD includes the similarity between the major histocompatibility complex class I chain-related gene-A (MICA) and HSP [4]. The expression of the cell membrane MICA molecule and secretion of its soluble form are increased during inflammatory stimuli. Autoreactive CD8 cytotoxic T cells to a peptide sequence derived from MICA, designated MICA-TM, can be detected in patients with BD only during the clinical active disease phase [5,6].

Two other autoimmune animal models of uveitis have been utilized extensively in BD research, the short peptides derived from self-proteins: retinal S-Antigen (S-Ag) and HLA-B27. The proposed mechanism is the induction of T cells bearing a TCR specifically directed to a short peptide sequence of the S-Ag defined as PDS -Ag. [7]. The cross-reactivity phenomenon is proposed in these two autoimmune animal models as there is some amino acid sequence homology between S-Ag and HLA-B27. Eventually the committed T cell line specifically directed to the PDS-Ag can bind to and become activated by a short peptide of the HLA-B27 molecule designated B-27PD.

It has been demonstrated that vaccination of rats with tropomyosin, a protein component of the contractile appara-

BD = Behçet's disease
HLA = human lymphocyte antigen
HSP = heat shock protein
TCR = T cell receptor

tus of muscles, causes a T cell-dependent autoimmune disorder including arthritis, dermatitis and uveitis [8]. Of the three short peptides derived from the TPM sequence, TPM 73-89 (designated T2) induced the highest inflammatory response in the immunized rats [8]. Moreover, excessive lymphoproliferative responses to TPM and its derivative peptides were noted in the sera of BD patients with uveitis [9].

Finally, in the HLA-B*5101 transgenic mice model, only hyperactivity of the neutrophils was demonstrated. HLA-B51 may present an endogenous antigen to cytotoxic T cells in these mice, thus activating constitutively the cytokine/chemokine cascades and causing superoxide release from neutrophils without a notable primary insult [10]. This B*5101 transgenic mice model did not develop any spontaneous clinical disease. However, it is not clear why.

More than two decades after the discovery by Ohno et al. [11] of the high prevalence of HLA-B51 in BD, the putative role of this molecule in the pathogenesis of BD is still obscure. The question whether antigens, which are extensively used in animal models for BD research, have the potential capacity to be bound to the HLA-B51 molecule remains unresolved. Immunoinformatics, a new sub-discipline of bioinformatics, addresses problems such as the crucial issue of epitope prediction [12]. The accurate prediction of peptide MHC binding provides a useful approach to candidate T cell epitope selection since it allows the number of experiments required for their identification to be minimized.

In the past three decades, an enormous number of 3-D structures of class I MHC molecules was determined, which led to tremendous progress in our understanding of the structure of the class I molecules and their binding affinity for ligands and TCR. All class I MHC molecules consist of two polypeptide chains – one heavy chain and the light chain β2-microglobulin. The binding groove of the class I MHC structure is divided into three domains, where α1 and α2 superdomains form a platform-like structure that potentially binds a ligand. The α3 domains are folded and hold the α1, α2 and the light chain together. Variation in the groove residues leads to the different binding specificities for their corresponding ligands [13].

The binding groove of class I MHC can encompass short peptides up to 9 amino acids long [14]. Database-driven models of peptide binding include multivariate methods based on amino acid sequence, 3-D configuration and polarity. Sequence motifs are currently still the best-known tools for predicting the peptide specificity of allele-dependent MHC-peptide binding. Motifs are characterized by a few dominant anchor positions with a very restricted set of allowed amino acids. Such anchors are considered essential for binding [15].

According to the hypothesis that the HLA-B*5101 allele has pathophysiological involvement in BD, the question arises: what are the potential ligands to this molecule? To cope with the enormous potential ligands we used several bioinformatics programs. These programs are designed to detect 9 mer aa sequences derived from the full-length proteins and to estimate the binding capacity of the HLA-B*5101 allele to those sequences derived from: HSP60, MICA, S-Ag, HLA-B27 and TPM. It is suggested that all these molecules are involved in the pathogenesis of BD or induce animal models of the disease. The answers to this question can minimize the list of potential autoantigens in BD, decrease markedly the need for traditional animal conformational experiments, and lead to a better understanding of the role of this histocompatibility receptor in the pathogenesis of BD.

MATERIALS AND METHODS

The comparison between the full sequences of all the proposed proteins as potential antigens for BD was conducted by: http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi.

Due to its steric structure the binding sites of HLA class I molecules can bind only short peptides with a restricted length of 9 ± 1 amino acids and require free N- and C-terminal ends [14]. We searched for 9-mer peptide candidates for antigenic peptide motifs derived from the antigens proposed to induce experimental BD in animals. For that purpose we used computerized programs that rank sequences of peptides according to their predicted half-time dissociation coefficient from the human HLA-B*5101 and its compatible rat MHC class I molecules designated MHC RT1.Al, by three programs: (http://www.bimas.dcrt.nih.gov/molbio/hla_bind/index.html), (http://meme.sdsc.edu. (MEME MASTversion 3.0), and (http://syfpeithi.bmi-heidelberg.com) [16].

RESULTS

A full-length comparison of the proteins revealed sequence homology between the HLA-B*5101 and MICA molecules (data not shown). The binding capacity of the human HLA-B*5101 and the rat class I molecule, MHC RT1.Al, to these proteins are depicted in Table 1.

It is evident that in most proteins tested, several short sequences with potential high binding capacity were found, but with exceptions: the rat molecule MHC RT1.Al has binding capacity motif to the short peptide B-27PD but HLA-B*5101 has no predictive binding capacity to this peptide; and the peptide designated PDS-Ag has a potential binding capacity to HLA-B*5101 but not to MHC RT1.Al.

It was found that the short peptide (aa sequence 73–89) derived from human TPM (73–89) molecule and defined as T2

TPM = tropomyosin
MHC = major histocompatibility complex

aa = amino acid

Table 1. Predicted binding capacity to human and rat class I molecules of peptides motif derived from the proposed antigens in animal models for BD

Protein	HLA B*5101	Rat MHC RT1.Al
HSP 65	**LAA**GVDPV	GVYEDL**LAA**
	SALQ**NAASI**	**NAAS**IAGLF
HSP65 (336-351)	**DAIAGRAVQ**	**DAIAGRAVQ**
	RVAQIRQEI	**RVAQIRQEI**
MICA	VAAA**AIFVI**	**AIFVI**IFTY
	EASEGNITV	HFVYDGELF
MICA-TM	AAAA**AIFVI**	**AIFVI**IFTY
HLA B*5101	**YAYDGKDYI**	**YAYDGKDYI**
	RAYLEGLCV	**RAYLEGLCV**
	YPAEITLTW	**YPAEITLTW**
Retinal S-Ag	EPIPVTVTV	*No Binding*
	LAVSLNKEI	*No Binding*
	LGELTSSEV	*No Binding*
PDS-Ag	**TSSEVATEV**	**TSSEVATEV**
	GELTSSEVA	**GELTSSEVA**
B27PD	*No Binding*	LSSWTAADT
h-TPM	DV**ASLNRRI**	**ASLNRRI**QL
	IAEDARKY	**IAEDARKY**
	TALQLEEA	**TALQLEEA**
h-TPM (73-89)= T_2	**DAEADVASL**	**DAEADVASL**

Bold characters designate identical amino-acids, Underline designates full sequence homology

Table 2. Predicted half-life disassociation of TPM derived peptides from the HLA-B*5101 molecule

T1			T2			T3		
aa	Subsequence	t½	aa	Subsequence	t½	aa	Subsequence	t½
7-15	LKLDKENAL	0.76	81-89	DAEADVASL	100	209-217	AEQAEKYSQ	1.1
5-13	QMLKLDKEN	0.19	78-86	KATDAEADV	60.5	201-208	TNNLKSLEA	0.1

T1 = TPM (5-21), T2 = TPM (73-89), T3 = TPM (201-217), aa = amino acid

has the highest predicted binding capacity to the human and rat class I molecules compared to the other TPM-derived peptides [Table 2]. These findings are in accordance with the clinical disease severity caused by TPM T2 in the animal model [8].

Sequence comparison analysis did not show any similarity between the above mentioned peptides to the recently published HLA class I-bound spectrum of peptides, defined by the authors as "peptidome" [17]. The "peptidome" contains 85 sequenced peptides eluted from the HLA class I molecules expressed on the human immunocyte cell line U937 [18]. Of the 85 reported peptides, 64 were eluted by exposing the U-937 cells to a mild acid; the other 21 peptides were identified by immunoprecipitation methods. From the 64 acid-eluted peptides 58 have predicted binding capacity to the rat MHC class I molecule (data not shown). All the 21 immunoprecipitated peptides have a binding capacity to the rat MHC class I molecule (data not shown).

DISCUSSION

It is commonly accepted that the T cells recognize a particular peptide presented by the HLA molecule. For that purpose, three elements are required. Firstly, TCR should have potential binding capacity to the amino acid sequences of the peptide. Secondly, the presented peptides should contain a particular motif that can be anchored by the HLA-binding sites and, finally, the HLA molecule should recognize the three-dimensional structure of the peptide.

The close association between BD and HLA-B51 in several ethnic groups [19] led to the speculation that HLA-B51 molecules are primarily involved in the development of BD via its capacity to present a foreign or self-antigen to specific CD8 TCR and activate them to become pathogenic T cells. This process culminates in the triggering of subsequent immunologic and inflammatory cascades leading to organ damage. In this study proteins that are utilized as antigens in animal models for BD were tested and were found to contain motif sequences with high predicted binding capacity to HLA-B*5101. Thus, this study implies that HLA-B51 has the potential to fulfill the second demand for functional immune response. Of great interest is the finding that the HLA-B51 molecule can recognize and bind its own sequence and its related molecule MICA. The question whether HLA-B51 is the key gene related to BD, or if some other genes in linkage disequilibrium with this molecule and the HLA-B51 serve only as a detectable marker for these pathogenic genes, could not be answered by this study. However, after three decades of intensive genetic research HLA-B51 is still considered an epidemiologically strongly associated genetic marker of BD [20,21].

The finding that the B-27PD molecule has no binding capacity motif to HLA-B*5101 could explain the observation that this peptide can lead to tolerance in the PDS-Ag uveitis model. On the other hand, B-27PD can serve as an inducer of animal disease possibly through binding to other receptors or populations of T cells. This raises the question whether this molecule is relevant only for BD.

The TPM model manifests a multi-organ involvement with resemblance to BD. This protein has many sequences with

predicted binding capacity to HLA-B*5101 [Table 1]. Of the three TPM-derived peptides, T2 demonstrated the highest predicted binding capacity to HLA-B*5101 and was found to be the most pathogenic in the animal model [8]. This finding strengthens the possibility that both TPM and HLA-B*5101 molecules may have some effect in the pathogenesis of BD.

Recently, Gebreselassie et al. [17] eluted and sequenced 85 peptides that were bound to the HLA class I molecules, presented on the outer membrane of the human histiocytic lymphoma cell line, and designated U-937. Utilizing two techniques, the weak acid dissociation yielded 64 peptides and immunoprecipitation with MHC class I antibodies identified 21 peptides. Only three identical peptides were identified by these two techniques [17]. This poor overlap between the techniques raises the question if these identified peptides are actually the full-scale human class I "peptidome." Of interest is their finding that only approximately 25% (21/85) of the peptides had a potential to be bound by the HLA-B*51 group of alleles [17]. The majority of bound peptides were eluted by the mild acid dissociation method, reflecting lower affinity between these peptides and the HLA-B*51 molecules. In accordance with the immunological paradigm that self-tolerance is actively maintained by clonal deletion of circulating lymphocytes bearing specific HLA peptides complexes [22], the authors concluded that the multiple low affinity peptides to the HLA-B*51 can avoid this physiologic process and might be involved in the generation of self-autoimmunity in BD. Moreover, this insight provides an indirect support to the hypothesis that the etiology of BD is a consequence of exposure of multiple antigens yet to be identified. These data are interesting and challenging since we could not identify – either in the eluted peptide or in the predicted proteins containing these sequences – structural similarity to proteins or peptides chosen due to their capacity to induce disease in rodents with features suggested to be related for BD. Explanations for this discrepancy are as follows: both technologies employed by Gebreselassie et al. to elute the peptidome are not sensitive enough, thus one can assume that other short peptides including those that served for our study were lost to detection. A contradictory possibility is that the antigens utilized for animal models of BD have a very high affinity to the HLA molecules. On the one hand these high affinity complexes could not be eluted; on the other hand they possess an unidentified pathological "escape mechanism" that prevents

the "forbidden clones" deletion of these lymphocytes and, consequently, initiates the chronic inflammatory process characterizing BD. Another possibility is that in BD patients the HLA-B51 molecule has different binding properties, thus their peptidome might be different from the peptidome represented by the U-937 cell line. This assumption merits a peptidome screening project of BD patients.

As for the cell line U-937 used to explore the HLA class I peptidone, one should remember that this is a malignant and immortal cell line. The U-937 cell line was derived by Sundström and Nilsson in 1976 [18] from malignant cells obtained from the pleural effusion of a patient with histiocytic lymphoma and was since propagated in vitro. The possibility that this patient had had BD concomitantly is unlikely. Such cell lines differ physiologically from normal and mature cells. Thus, the results gained by utilizing such a cell line can be misleading once extrapolation is employed on a different pathophysiologic process as seen in BD.

Further studies are warranted of HLA-B51 transgenic mice immunized with full length HSP, MICA, S-Ag, HLA-B27, TPM, HLA-B*5101, whose peptides were predicted in the present study as well as those eluted and sequenced by Gebreselassie et al. [17]. Moreover, peptidome analysis of large groups of BD patients from different ethnic populations is clearly needed in order to understand their precise role in the pathogenesis of this disease.

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Capsule

Osteoprotection by semaphorin 3A

The bony skeleton is maintained by local factors that regulate bone-forming osteoblasts and bone-resorbing osteoclasts, in addition to hormonal activity. Osteoprotegerin protects bone by inhibiting osteoclastic bone resorption, but no factor has yet been identified as a local determinant of bone mass that regulates both osteoclasts and osteoblasts. Hayashi and team show that semaphorin 3A (Sema3A) exerts an osteoprotective effect by both suppressing osteoclastic bone resorption and increasing osteoblastic bone formation. The binding of Sema3A to neuropilin-1 (Nrp1) inhibited receptor activator of nuclear factor-κB ligand (RANKL)-induced osteoclast differentiation by inhibiting the immunoreceptor tyrosine-based activation motif (ITAM) and RhoA signaling pathways. In addition, Sema3A and Nrp1 binding stimulated osteoblast and inhibited adipocyte differentiation through the canonical Wnt/β-catenin signaling pathway. The osteopenic phenotype in *Sema3a*$^{-/-}$ mice was recapitulated by mice in which the Sema3A-binding site of Nrp1 had been genetically disrupted. Intravenous Sema3A administration in mice increased bone volume and expedited bone regeneration. Thus, Sema3A is a promising new therapeutic agent in bone and joint diseases.

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Eitan Israeli

Capsule

Pathogen-induced human T_H17 cells produce IFNγ or IL-10 and are regulated by IL-1β

Interleukin (IL)-17-producing CD4+ T helper cells (TH17) have been extensively investigated in mouse models of autoimmunity. However, the requirements for differentiation and the properties of pathogen-induced human TH17 cells remain poorly defined. Using an approach that combines the in vitro priming of naive T cells with the ex vivo analysis of memory T cells, Zielinski et al. describe two types of human TH17 cells with distinct effector function and differentiation requirements. Candida albicans-specific TH17 cells produced IL-17 and interferon-gamma (IFNγ), but no IL-10, whereas Staphylococcus aureus-specific TH17 cells produced IL-17 and could produce IL-10 upon restimulation. IL-6, IL-23 and IL-1β contributed to TH17 differentiation induced by both pathogens, but IL-1β was essential in C. albicans-induced TH17 differentiation to counteract the inhibitory activity of IL-12 and to prime IL-17/IFNγ double-producing cells. In addition, IL-1β inhibited IL-10 production in differentiating and in memory TH17 cells, whereas blockade of IL-1β in vivo led to increased IL-10 production by memory TH17 cells. We also show that, after restimulation, TH17 cells transiently downregulated IL-17 production through a mechanism that involved IL-2-induced activation of STAT5 and decreased expression of ROR-γt. Taken together these findings demonstrate that by eliciting different cytokines, C. albicans and S. aureus prime TH17 cells that produce either IFNγ or IL-10, and identify IL-1β and IL-2 as pro- and anti-inflammatory regulators of TH17 cells both at priming and in the effector phase.

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Eitan Israeli

If you write to impress it will always be bad, but if you write to express it will be good

Thornton Wilder (1897-1975), American playwright and novelist who won three Pulitzer prizes