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Strong Association of HLA-B27 Heavy Chain With β_2-Microglobulin

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ABSTRACT: Monoclonal antibody TG1 recognizes specifically antigens HLA-B27, B7, B22 and B17 on cell surface in cytotoxicity and cytofluorometry tests. When cell detergent extracts were subjected to SDS PAGE under mild conditions (no heating and no reduction of the sample) followed by Western blotting, TG1 detected exclusively a complex of B27 heavy chains with β_2-microglobulin (as a 50 kDa band) whereas the other B-locus antigens (B7, B22, B17) were detected as free 43 kDa heavy chains under the same conditions. When the samples were boiled prior to SDS PAGE, TG1 detected again the 43 kDa free heavy chains of B7, B22 and B17 but no zone corresponding to B27 could be detected indicating that the epitope in free B27 chains is more sensitive to denaturation by SDS. Thus, our main finding is that the interaction of HLA-B27 heavy chain with β_2-microglobulin appears to be stronger than that of the other HLA-B chains. The resistance of the HLA-B27/β_2-microglobulin complex to the SDS dissociation is strikingly similar to the behavior of MHC class II molecules under similar conditions. Thus, it may be speculated that HLA-B27 complexes can be also more stable than other MHC class I molecules under more physiological dissociative conditions (e.g. in endosomal compartments). This feature might potentially influence antigen presentation by HLA-B27 and contribute to the well known disease linkage of HLA-B27. *Human Immunology 61, 1197–1201 (2000).* © American Society for Histocompatibility and Immunogenetics, 2000. Published by Elsevier Science Inc.

KEYWORDS: HLA antigens; β_2-microglobulin; Western blotting; monoclonal antibody.

ABBREVIATIONS

HLA	Human leukocyte antigen	McAb	Monoclonal antibody
b2m	β_2-microglobulin	SDS	Sodium dodecyl sulfate
MHC	major histocompatibility complex	PBL	Peripheral blood lymphocyte

INTRODUCTION

The major histocompatibility complex (MHC) class I molecules are composed of two non-covalently associated polypeptide chains, the heavy highly polymorphic α chain (43 kDa) and the monomorphic light chain β_2-microglobulin (b2m; 12 kDa). The main function of these molecules is to bind short antigenic peptides derived from intracellular proteins and present them to $CD8^+$ cytotoxic T lymphocytes [1]. Complexes of MHC class I molecules with peptides are also recognized by several types of inhibitory and activating receptors of NK cells [2]. Some allelic forms of human MHC class I molecules (HLA) are genetically associated with certain diseases. Thus, individuals carrying HLA-B27 are at an increased risk of developing ankylosing spondylitis, acute anterior uveitis and reactive arthritis [3]. Although it is broadly believed that the mechanism responsible for the role of B27 in disease susceptibility is somehow

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related to the peptide(s) presented by this molecule, the real mechanism by which HLA-B27 is responsible for the disease association remains unclear [3–6]. Certainly the explanation of the HLA-B27 disease association must be somehow connected with the unique features of the B27 molecule itself (unless B27 is only the marker of a closely linked, disease susceptibility gene). In the present study we describe a new feature of the B27 molecule as detected by McAb TG1 specifically recognizing cell surface expressed antigens B27, B7, B22 and B17, i.e. the B27-cross reacting antigens (CREG) and B17.

MHC class I, in contrast to MHC class II molecules, are readily dissociated into their subunits (α chains and b2m) by exposure to the anionic detergent SDS, even without heating the sample and in absence of any reducing agent. Thus, upon SDS PAGE of cell membranes solubilized under these conditions, α chains and b2m migrate separately and essentially no α/b2m complexes are observed. In contrast, most MHC class II molecules are dissociated into their constituent α and β chains only upon heating (boiling) in the presence of SDS; without heating, most MHC class II molecules migrate as αβ dimers on SDS PAGE [7, 8] which can be subsequently easily detected by Western blotting.

In the present study we demonstrate that the B27/b2m complex is strikingly more resistant to dissociation by SDS as compared to the other HLA-B antigens recognized by the TG1 McAb. In this respect, the B27/b2m resembles rather the MHC class II molecules.

MATERIALS AND METHODS

Cells

Peripheral blood lymphocytes (PBL) were obtained from blood donors and were HLA-typed by a series of HLA specific typing sera using the microlymphocytotoxicity test (classical NIH technique). The mutant lymphoblastoid cell line LCL 721.221 (MHC class I negative) stable transfected with HLA-B*2705 was obtained from Dr. L. Pazmany (Harvard University, Cambridge, MA), mouse fibroblast cell line transfected with human b2m gene together with gene encoding the HLA-B7 (line 8024) was prepared and characterized by one of us (M.P.). Spleen cells from C57B1/10 (B10) mice transgenic for both human HLA-B*2702 and b2m gene [9] were used in some experiments; non-transgenic littermates were used as controls.

Antibodies

The hybridoma producing McAb TG1 was prepared from a B10 mouse transgenic for human b2m immunized with splenocytes of a B10 mouse double transgenic for HLA-B27 and human b2m [9]. Ten days after six biweekly i.p. injections, splenocytes of the recipient mouse were fused with myeloma cells, hybridomas secreting antibodies reactive with B27-positive cells were selected and cloned using standard techniques. McAb TG1 (IgG2a) was strongly complement-dependent cytotoxic; using standard cytofluorometric tests, it bound well to lymphocytes of the B27-transgenic mice but not to those of human b2m-transgenic or non-transgenic mice. It bound strongly to human PBL expressing HLA-B27, B7 and B22 and less strongly to HLA-B17$^+$ PBL as determined by cytoxicity tests and cytofluorometry but not to PBL not expressing any of these HLA-B molecules (P. Ivanyi, unpublished); this reaction pattern was amply confirmed by testing of this McAb on extensive panels of human cells of various populations during the 11th International HLA Histocompatibility Testing Workshop [10]. TG1 recognizes equally well B27.05 and B27.02 subtypes (P. Ivanyi, unpublished). TG1 McAb purified from ascitic fluid by Protein A affinity chromatography was used throughout this study. McAb B2M-02 (mouse IgG1, reactive with human b2m) was previously prepared and characterized in the laboratory of one of us (V.H.).

SDS PAGE and Western Blotting

2.5 million cells were lysed for 1 h on ice in 100 μl of isotonic lysis buffer (0.15 M NaCl, 10 mM Tris pH 8.0) containing 1% detergent CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate) and protease inhibitors (iodoacetamide (0.1 mM), aprotinin (5 μg/ml), leupeptin (10 μg/ml), pepstatin A (10 μg/ml), EDTA (5 mM), phenylmethyl sulfonylfluoride (1 mM)). Nuclei and other insoluble materials were removed by centrifugation. The supernatant was mixed 1:1 at room temperature with the standard 2× concentrated non-reducing SDS-sample buffer solution (i.e. final concentration of SDS in the electrophoretic sample was 2%). The samples were either kept at room temperature or heated for 3 min on boiling water bath and then run on 10% polyacrylamide gel using the standard Laemmli buffer system. Following electrophoresis the samples were electroblotted onto Immobilon-P membrane (Millipore) and immunoperoxidase stained by McAbs and anti-mouse immunoglobulin horseradish peroxidase conjugate (Bio-Rad) as the secondary reagent; the Enhanced Chemiluminescence (ECL) detection reagent (Amersham) was used for luminographic visualization.

RESULTS AND DISCUSSION

The samples of detergent-solubilized proteins prepared as described above from PBL of 15 blood donors were analyzed by SDS PAGE and Western blotting. As shown in Fig. 1 and Tab. 1, TG1 detected a 43 kDa zone corresponding to free α chains in both boiled and non-

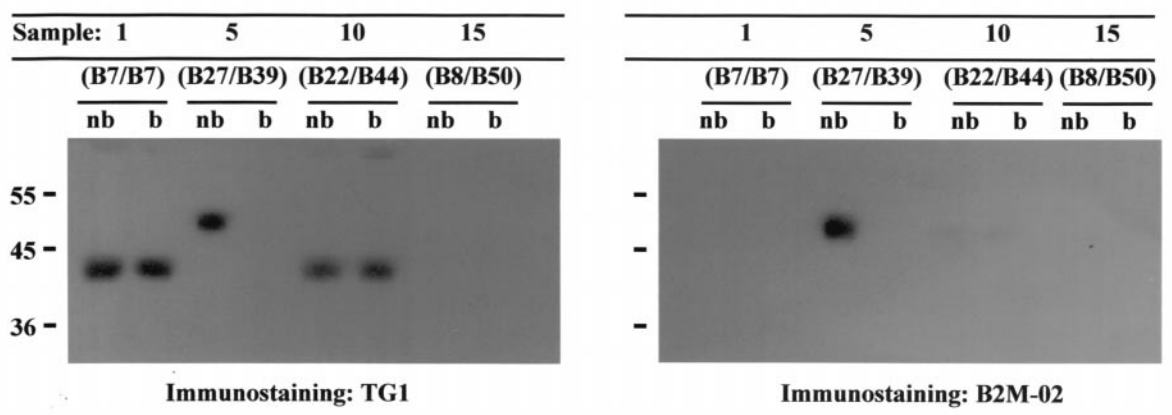

FIGURE 1 Western blotting of four representative samples. Sample numbers at the top correspond to those in Table 1. The HLA-B antigens expressed in the respective cells are indicated in the header. Non-reduced non-boiled (nb) or boiled (b) samples were run on 10% gel and the electroblotted molecules were detected either by TG1 or B2M-02, as indicated. Positions of mol. wt. standards (in kDa) are shown at left margin.

boiled samples containing the TG1-reactive allelic forms B7, B22 and B17 but, as expected, not in samples containing only non-TG1 reactive allelic forms (B62, B60, B8, B50). Surprisingly, in all non-boiled samples containing B27, a 50 kDa zone was stained with TG1; the same zone was stained also with the anti-b2m McAb indicating that it corresponded to the B27/b2m complex (true mol. wt. of the complex is 55 kDa but obviously the incompletely denatured, relatively compact complex exhibited electrophoretic mobility corresponding to apparently smaller size). Notably, in the three samples containing B27 as the only TG1-reactive molecule (No. 4, 5 and 6 in Tab. 1) only this 50 kDa species could be detected by TG1 (and B2M-02) while no zone could be detected in the boiled samples. The same phenomenon, i.e. presence of the single 50 kDa zone detected by TG1 and B2M-02 in non-boiled samples and absence of any zone recognized by TG1 in the boiled samples, was observed also when we similarly analyzed a human transfectant cell line (721.221-B27) expressing the complex of B27 and human b2m as the only MHC class I molecule or splenocytes of B27 + b2m transgenic mice (Fig. 2), while B7 transfectants (line 8024) yielded exclusively the 43 kDa b2m-free zone in both non-boiled and boiled samples (data not shown). The 50 kDa zone in the non-boiled sample of the splenocytes of B27 + human

TABLE 1 Reaction pattern of mAbs TG1 and B2M-02 on Western blots of a panel of PBL

Sample Number	HLA type						Not boiled				Boiled			
							50 kDa band		43 kDa band		50 kDa band		43 kDa band	
	A	A	B	B	C	C	TG1	B2M-02	TG1	B2M-02	TG1	B2M-02	TG1	B2M-02
1	2	3	**7**	7			−	−	+	−	−	−	+	−
2	3		**7**	14	7		−	−	+	−	−	−	+	−
3	3	29	**7**	13			−	−	+	−	−	−	+	−
4	2	3	**27**	50	2	6	+	+	−	−	−	−	−	−
5	2		**27**	39	2		+	+	−	−	−	−	−	−
6	2		**27.05**	44	3	5	+	+	−	−	−	−	−	−
7	3	26	**27**	7	2	7	+	+	+	−	−	−	+	−
8	2	28	**27.05**	7	2		+	+	+	−	−	−	+	−
9	69	33	**22**	21	3	7	−	−	+	−	−	−	+	−
10	2	29	**22(55)**	44	3		−	−	+	−	−	−	+	−
11	3	24	**22(55)**	40	3		−	−	+	−	−	−	+	−
12	1	24	**17(57)**	8	6	7	−	−	+	−	−	−	+	−
13	2		**17(57)**	60	3	6	−	−	+	−	−	−	+	−
14	2	11	62	60	3		−	−	−	−	−	−	−	−
15	1	11	8	50	6		−	−	−	−	−	−	−	−

HLA-B-locus antigens detected by McAb TG1 are in bold. B55 and B57 are subtypes of B22 and B17, respectively. C-locus typing of some cells is incomplete.

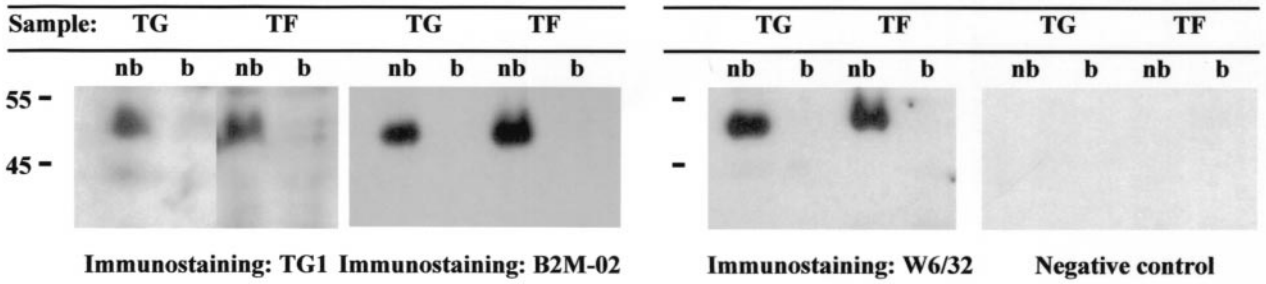

FIGURE 2 Western blotting of B27 + human b2m transgenic mouse cells (TG) and human lymphoblastoid 721.221-B27 transfectant cell line (TF). Non-reduced non-boiled (nb) or boiled (b) samples were analyzed as in Fig. 1 and the blots were immunostained by the McAb given at the bottom; the negative control represents an irrelevant McAb.

b2m transgenic mice was recognized also by the McAb W6/32 which is thought to recognize only intact complexes of MHC class I molecules with b2m (Fig. 2). Taken together, McAb TG1 reacts specifically with native, cell surface B27, B7, B22 and B17 molecules. However, under the conditions of Western blotting this mAb recognizes free α chains of B7, B22 and B17 dissociated from b2m under relatively mild conditions (exposure to SDS at room temperature) while it reacts only with the complex B27/b2m uniquely resistant to dissociation by SDS at low temperature. Interestingly, TG1 does not react with free B27 chains dissociated from b2m under more harsh conditions (boiling in the presence of SDS), while it does react with the other free α chains (B7, B22, B17) exposed to such more denaturative conditions.

The major finding reported here is that the complex B27/b2m is apparently more resistant to SDS-induced dissociation than other HLA-B molecules, reflecting probably an unusually strong interaction between the B27 chain and b2m. This could be either due to inherent structural features of the B27 polypeptide chain influencing the strength of interaction with b2m or due the nature of the peptides bound to B27 which may specifically stabilize the whole assembly B27/b2m/peptide [11, 12]. An intriguing possibility is that only a fraction of HLA-B27 molecules containing a specific peptide(s) is SDS-resistant (and the putative SDS-sensitive HLA-B27 molecules are "invisible" to TG1 because it does not bind with sufficient affinity to free B27 chains) and that this fraction may have unique biological properties possibly related to the known disease linkage of B27. The stronger association with b2m may be another molecular peculiarity of B27, in addition to its recently described capacity to form disulfide-linked homodimers of heavy chains [13].

The resistance of the B27/b2m complex to SDS is reminiscent of the well known resistance of MHC class II-peptide complexes to similar treatments [7, 8]. Although the system used in our present study can be considered as highly artificial, the striking similarity of B27 to MHC class II molecules under these conditions may indicate also a physiological relevance. It can be speculated that the B27/b2m complexes may be, in analogy to MHC class II, also more resistant to dissociation and degradation in endosomal compartments. Thus, some aspects of antigen presentation by B27 may be, in contrast to other MHC class I molecules, partially analogous to MHC class II.

In the present study we were able to examine only a limited number of MHC class I allelic forms (B27, B7, B22 and B17) as to their resistance to SDS treatment. Actually, it should be noted that the absence of the 50 kDa zone detected by the McAb to b2m in any of the non-B27 samples shown in Table 1 indicates that also the other MHC class I molecules (e.g. A1, A2, A3, A11, A24, A26, A28, A29, A33, B8, B13, B14, B21, B40, B44, B50, B60, B62, C2, C3, C6, C7) are sensitive to SDS treatment. It remains to be determined how unique is this SDS-resistance for HLA-B27 among all MHC class I molecules and what fraction of total HLA-B27 actually exhibits such a strong, SDS-resistant interaction with b2m, and to what extent is this phenomenon dependent on the identity of the peptide(s) bound to HLA-B27. Such studies, currently in progress in our laboratories, require a set of antibodies reactive with various forms of native, partially and completely denatured HLA-A, -B and -C molecules as well as a panel of well defined transfectants. Preliminary results of these studies indicate that the SDS resistance is indeed limited to only very few MHC class I allelic forms (unpublished data).

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