induce any changes in recipient mice. It seemed unlikely that the adoptive transfer of contaminating granulocytes was responsible for the granulocyte abnormality, because mice injected with mutant spleen cells containing >90% granulocytes did not develop an abnormal phenotype in any cell lineage. However, the expression of IL-2Rβ on myeloid cells necessitates cautious interpretation of these data; we cannot yet exclude the possibility that receptor deficiency may play some direct role in the observed myeloid disorder.

Taken together, our results suggest that IL-2Rβ, in addition to its known role as a mediator of activation signals for T-cell proliferation, is also a central component of the regulatory system in T cells. It appears that a complete lack of signaling through the IL-2Rβ chain can result in deregulation of these lymphocytes and consequent loss of homeostasis and autoimmunity.

REFERENCES AND NOTES

1 W. J. Leonard et al., Nature 311, 626 (1984); T. Negoro et al., ibid. 351, 631; D. Cosman et al., ibid. 312, 768 (1984); M. Hattori et al., ibid. 244, 261 (1989); T. Takeshita et al., ibid. 267, 379 (1992).


Identification of a Graft Versus Host Disease–Associated Human Minor Histocompatibility Antigen

Joke M. M. den Haan, Nicholas E. Sherman, Els Blokland, Eric Huczko, Frits Koning, Jan Wouter Drijfhout, Jonathan Skipper, Jeffrey Shabanowitz, Donald F. Hunt, Victor H. Engelhard, Els Goumy*

Minor histocompatibility antigen disparities between human leukocyte antigen (HLA)-matched bone marrow donors and recipients are a major risk factor for graft versus host disease (GVHD). An HLA-A2.1-restricted cytotoxic T-cell clone that recognized the minor histocompatibility antigen HA-2 was previously isolated from a patient with severe GVHD after HLA-identical bone marrow transplantation. The HLA-A2.1-bound peptide representing HA-2 has now been identified. This peptide appears to originate from a member of the non-filament-forming class I myosin family. Because HA-2 has a phenotype frequency of 95 percent in the HLA-A2.1-positive population, it is a candidate for immunotherapeutic intervention in bone marrow transplantation.

In the 1970s, human bone marrow transplantation (BMT) became available as a therapy for severe aplastic anemia, leukemia, and immune deficiency disease (1). The long-term results of allogeneic BMT have greatly improved for a variety of reasons, including the participation of HLA-matched siblings as marrow donors, advancements in pretransplant chemoradiotherapy, the prophylactic use of potent immunosuppressive drugs, and better antibiotics and isolation procedures. Nonetheless, the selection of major histocompatibility complex (MHC)-identical donors and recipients does not guarantee avoidance of GVHD, nor does it ensure disease-free survival, even when the donor and the recipient are closely related (2). Up to 80% of cases of GVHD result from allogeneic BMT between unrelated HLA-matched adults (3, 4). Disparities in minor histocompatibility antigens (mHags) between the donor and recipient constitute a significant risk for graft failure or GVHD. These conditions necessitate lifelong pharmacological immunosuppression of organ and BMT recipients.

Cytotoxic T-lymphocytes (CTLs) that are specific for host mHags have been detected in patients who develop GVHD after BMT from donors who are genotypically HLA-identical (7). Immunogenetic analyses with CTL clones have identified five non-sex-linked mHags, designated HA-1 through -5, that are recognized in a classical MHC-restricted fashion (8) and are products of single genes that segregate in a Mendelian fashion (9). In a prospective study, mismatching of these mHags significantly correlated with GVHD (6). These mHags show defined phenotype frequencies (8) and tissue distribution patterns (10). HA-1 and HA-2 are uniquely expressed on hematopoietic-derived cells, including leukemic cells (11), whereas HA-3 and HA-4 are present on other cell types as well (10).

Two unusual mHags have been character-
capillary HPLC. The effluent was split with an on-line splitter (18) and was directed simultaneously into the mass spectrometer and into a 96-well microtiter plate for a subsequent \(^{51}\)Cr release assay. In this way, mass spectrometry data on individual peptides could be correlated with epitope recon-
stition activity. Figure 2A shows a single peak of HA-2-sensitizing activity in wells 50 to 53. Of the many peptides present in these wells (Fig 2B), the relative ion abundance profile of five [with mass-to-charge ratios (m/z) of 651, 869, 965, 979, and 1000] matched the activity profile of the HA-2-specific CTL-sensitizing activity (Fig 2A). Collision-activated dissociation (CAD) analysis performed for the species with m/z of 979 revealed the existence of two different peptides, YIGEVLVSV and SXDFGTXQV (19) (Fig 3, A and B). The X represents L or I, which cannot be distinguished by mass spectrometry under these conditions. We next made synthetic peptide mixtures with an equimolar mixture of L and I in place of X and assayed them for HA-2-specific CTL-sensitizing activity. Only incubation with peptide mixture YIGEVLVSV resulted in lysis of T2 (17, 20).

To identify the naturally processed peptide epitope, we synthesized four peptides with L or I at positions 2 and 6. Microcapillary HPLC coelution studies revealed that three of these synthetic peptides (YIGEVLVSV, YLGEVLVSV, and YLGEVIVSV) coeluted with the naturally processed peptide, whereas peptide YIGEVLVSV did not (17). These three peptides could not be distinguished from one another or from the naturally processed peptide under several different HPLC elution conditions. All three peptides sensitized the T2 cell line for lysis by clone 5H17 (Fig 4A). Peptide YIGEVLVSV sensitized targets for half-maximal lysis at a concentration of 40 nM, whereas the sensitizing concentrations for peptides YLGEVLVSV and YLGEVIVSV were substantially higher (1.5 and 2.25 nM, respectively). These concentrations were within the range of 10 pM to 50 nM established for other naturally processed epitopes (18, 21). Panel analysis indicates that clone 5H13 is an independently derived CTL that also recognizes HA-2 but differs slightly from clone 5H17 in its fine specificity of antigen recognition (8, 22). Clone 5H13 also recognized all three peptide variants (Fig 4B). Although the concentration of peptides necessary to give half-maximal epitope recognition was 5 to 10 times higher than for clone 5H17, peptide YIGEVLVSV still sensitized at one-hundredth the concentration of the other two. Thus, despite their fine specificity differences, both HA-2-specific CTL clones recognized the same peptide epitope.

Binding studies with these three peptides showed that peptide YIGEVLVSV also had the highest binding affinity for HLA A2 1. The concentration of YIGEVLVSV that inhibited the binding of the iodinated standard peptide to purified HLA A2 1 by 50% (IC50) was 67 nM, whereas the IC50 values for YLGEVLVSV and YLGEVIVSV were 17 and 27 nM, respectively (Fig 5). These values place these peptides among the highest-affinity naturally processed peptides that have been identified to date (23). However, although there is less than a fourfold difference in binding affinities among these three peptides, peptide YIGEVLVSV sensitizes target cells for recognition by clones 5H17 and 5H13 at one-fiftieth to one-hundredth the concentration of the other two. This finding indicates that peptide YIGEVLVSV is recognized with the highest affinity by the T cell receptors of both of these CTL clones, and that it is the actual HA-2 epitope.

To determine the quantity of HA-2 peptide present on EBV-BLCLs, we compared a positive HPLC fraction with a known amount of synthetic peptide on the mass spectrometer. Assuming an overall purification yield of 12% (18), we estimated that 260 HA-2 peptide–HLA-A2 complexes were expressed per cell. This number is similar to values determined for other sequenced T cell epitopes (18, 21), and it indicates that HA-2 is moderately abundant compared with other naturally processed peptides (18, 24).

A search of DNA and protein sequence databases identified six distinct coding sequences that match peptide YIGEVLVSV at eight of nine residues, and an additional seven that match at seven of nine residues. All of these sequences are derived from non-
filamentous class I myosins, a large family of proteins that are involved in cell locomotion and organelle transport (25, 26). Most mismatches to the HA-2 sequence occurred at peptide position 4. Nonetheless, at least two class I myosin sequences contain a glutamic acid at this position. The closest known human sequence corresponds to residues 51 to 59 from unconventional myosin IC (YIGSVLISV), which is also the only completely sequenced class I myosin gene (27). However, although different cell types appear to express multiple class I myosins simultaneously, tissue-restricted expression of class I myosins has also been reported (26, 28). Because the total number of family members is still not known, the HA-2 peptide epitope most likely derived from an as yet uncharacterized human class I myosin protein.

Virtually all of the known class I myosin sequences, regardless of their species of origin, show identity at six or more of the nine positions within the sequence that corresponds to the HA-2 peptide, and these sequences also contain an isoleucine at position 2 and a valine at position 9. These latter residues represent the most important elements of the HLA-A2.1-binding motif (23, 29). Thus, the basis for differential expression of the mHag HA-2 within the human population may be allelic polymorphism that results in presentation of homologous but nonidentical peptides from this region by HLA-A2.1 molecules, or in the failure to present a peptide that has lost one of the motif residues. However, the large amount of HA-2–related sequence conservation among different class I myosins from the same species also suggests that several peptides related to HA-2 may be expressed in association with HLA-A2.1 in a single individual and may be cross-reactively recognized by HA-2–specific T cells. Indeed, the human class IC myosin–derived peptide YIGSVLISV was able to sensitize T2 cells for lysis by clones 5H17 and 5H13 and could induce 50% lysis at concentrations of 5 to 50 nM (17). Because these peptides could also exert a profound tolerogenic effect on the HA-2–specific T cell repertoire, an alternative hypothesis is that a polymorphism in the class I antigen processing system results in a failure to express all or many members of this set of peptides in HA-2–negative individuals. Finally, because HA-2 is only presented by hematopoietic cells, either the expression of its unknown class I myosin source protein is tissue-specific or HA-2 is only presented as a consequence of tissue-specific processing.

Until now, information on mHags has been scarce. Although the physiological function of mHags is still unknown, their pivotal role in organ transplantation in general, and in BMT in particular, is undeniable. The availability of the mHag peptide sequence may allow in vivo modification of GVHD–related T cell responses. Because the mHag HA-2 is expressed on cells of the hematopoietic lineage, including leukemic cells, it is a candidate for immunotherapy for leukemia before BMT.

REFERENCES AND NOTES
1 M. M. Borton, Transplantation 9, 571 (1970)
4 P. G. Beatty et al., Transplantation 51, 443 (1991)
5 P. J. Martin, Bone Marrow Transplant 8, 217 (1991)
6 E. Goulmi, unpublished results
8 C. van Ellis et al., Immunogenetics 35, 161 (1992)
9 S. Schreuder et al., ibid. 38, 80 (1993)
12 D. van der Harst et al., Blood 84, 1060 (1994)
15 The HA-2–specific CTL clone 5H17 originated from a female patient who underwent BMT for severe aplastic anemia. The pretransplant conditioning regime consisted of total lymphoid irradiation and cyclophosphamide. The patient was grafted with non-T cell-depleted bone marrow from her HLA-identical
Assessment of microbial population growth rate in aquatic systems is difficult because ubiquitous grazers remove cells as quickly as they are produced. If the cell cycle of a population is in phase with the daily light cycle, cell division rates can be estimated from changes in the fractions of cells in each cell cycle stage (1) during the light cycle, cell division rates can be estimated from changes in the fractions of cells in each cell cycle stage (1) during the light cycle. DNA replication occurred in the afternoon and cell division occurred at night. Growth rates were maximal (about one doubling per day) at 30 meters and decreased toward the surface and the bottom of the ocean. Estimated Prochlorococcus production varied between 174 and 498 milligrams per square meter per day and accounted for 5 to 19 percent of total gross primary production at the equator. Because Prochlorococcus multiplies close to its maximum possible rate, it is probably not severely nutrient-limited in this region of the oceans.

Prochlorococcus decreased in abundance but was present down to 150 m (1 April, Fig. 1). Flow cytometric DNA histograms displayed a single peak composed of cells in the G$_1$ phase of the cell cycle (3) during the late hours of night and in the early morning (Fig. 2). Around 1400 (arrowhead, Fig. 2), a distinct population of cells began to enter the S phase at 30 m and below, while cells in the surface layer (0 to 15 m) remained in G$_0$. By 1700, a large fraction of cells in the deeper samples had finished genomic DNA replication and entered the G$_2$ phase. Meanwhile, in the surface layer (0 and 15 m), cells had entered the S phase. By 2300, cells at all depths had divided and reentered the S phase at 30 m and below, while cells in the surface layer (0 to 15 m) remained in G$_0$. By 1700, a large fraction of cells in the deeper samples had finished genomic DNA replication and entered the G$_2$ phase. Meanwhile, in the surface layer (0 and 15 m), cells had entered the S phase. By 2300, cells at all depths had divided and reentered the S phase.

Growth of Prochlorococcus, a Photosynthetic Prokaryote, in the Equatorial Pacific Ocean

Daniel Vaulot,* Dominique Marie, Robert J. Olson, Sallie W. Chisholm

The cell cycle of Prochlorococcus, a prokaryote that accounts for a sizable fraction of the photosynthetic biomass in the eastern equatorial Pacific, progressed in phase with the daily light cycle. DNA replication occurred in the afternoon and cell division occurred at night. Growth rates were maximal (about one doubling per day) at 30 meters and decreased toward the surface and the bottom of the ocean. Estimated Prochlorococcus production varied between 174 and 498 milligrams per square meter per day and accounted for 5 to 19 percent of total gross primary production at the equator. Because Prochlorococcus multiplies close to its maximum possible rate, it is probably not severely nutrient-limited in this region of the oceans.