A Gene Required for Class II–restricted Antigen Presentation Maps to the Major Histocompatibility Complex

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Summary

We have previously described a set of mutants (16.23-selected mutants) of a B lymphoblastoid cell line that are defective in the presentation of intact proteins to class II–restricted T cells, but effectively present immunogenic peptides. The mutations in these mutants are recessive in somatic cell hybrids and are not in Class II structural genes. Here, we report on a unique mutant, 5.2.4, in which a similar defect in class II–restricted antigen presentation has occurred in association with a one-megabase homozygous deletion in the class II region of the major histocompatibility complex (MHC). The defects in class II presentation among three of the 16.23-selected mutants, and between these mutants and 5.2.4, are noncomplementary in somatic cell hybrids. This suggests that the class II presentation-defective phenotype in all four mutants results from lesions in a single MHC-linked gene, a conclusion strengthened by the finding that in a hybrid made with a second, unrelated MHC deletion mutant, T2, the class II presentation defect in a 16.23-selected mutant is also not complemented. Mutant 5.2.4, in addition to its class II presentation defect, is also defective in surface expression of MHC class I molecules, most likely because its deletion encompasses the peptide supply factor 1 gene, whose function is known to be required for normal abundance of cell surface class I molecules. However, the surface abundance of class I molecules is normal in the 16.23-selected mutants, suggesting that the lesions affecting class I surface abundance and class II presentation result from mutations in different genes.

Presentation of an endocytosed protein antigen by APC requires that it be processed intracellularly into immunogenic peptide fragments, which become bound to MHC class II molecules (reviewed in reference 1). The complex of peptide and MHC molecule is transported to the APC surface, where it can be recognized by $CD4^+$ T cells. This handling of foreign antigen is apparently part of a more general process in which class II molecules en route to the cell surface bind both foreign and self peptides. The majority of cell surface class II molecules are occupied by self-peptides (2). Nonetheless, this system provides effective immune surveillance of the extracellular space, because occupancy of a small fraction of MHC class II molecules with immunogenic peptide is sufficient to activate specific T cells (3, 4).

Previously, we used an anti–HLA-DR3 mAb, 16.23, to select a set of B-lymphoblastoid mutants (hereafter called 16.23-selected mutants) which define a novel step in class II–restricted antigen presentation (5, 6). Unlike the progenitor cells, the mutants, exemplified by mutant 9.5.3, are unable to stimulate specific T cell clones when exposed to intact protein antigens. Their class II molecules effectively present exogenously supplied peptides, however. Thus, the mutants appear unable to form intracellular MHC class II–peptide complexes from intact protein antigens. The class II structural genes and the surface abundance of class II molecules in these mutants are normal. However, the mutants demonstrate a subtle change in class II molecule conformation. Their HLA-DR3 molecules have lost expression of the epitope recognized by antibody 16.23, and class II dimers extracted from the mutants dissociate into monomers in SDS-PAGE, whereas dimers extracted from progenitor cells under the same conditions do not dissociate. We have proposed that these conformational changes are related to underoccupancy of cell surface class II molecules resulting from defective peptide loading during class II molecule biosynthesis. The amount of invariant chain (Ii),[1] its glycosylation, and the kinetics of its association and dissociation from DR molecules do not differ in mutant 9.5.3 and its progenitor, 8.1.6 (reference 6 and E.

[1] *Abbreviations used in this paper:* B-LCL, B lymphoblastoid cell line; EMS, ethylmethane sulfonate; HBsAg, hepatitis B surface antigen; Ii, invariant chain; PSF1, peptide supply factor 1; TT, tetanus toxin/toxoid.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/91/12/1607/09 $2.00
Volume 174 December 1991 1607–1615

Mellins, unpublished data). These findings suggest that the DR-Ii complexes in the mutants traffic normally through the Golgi apparatus to the compartment where Ii dissociation occurs. The phenotypic features of the mutants thus implicate gene products other than class II molecules and Ii in the formation of MHC class II–peptide complexes.

Here, we report on an informative new mutant B lymphoblastoid cell line (B-LCL), 5.2.4, which was selected with an anti-DR mAb for loss of surface expression of DR molecules. In contrast to the 16.23-selected mutants, which are likely to be point mutants (reference 7 and D. Pious, unpublished data), mutant 5.2.4 has a homozygous deletion within the class II region of the MHC. Mutant 5.2.4 resembles the unrelated mutant human cell lines, 721.174 and T2 (8–10), and the mutant murine line RMA-S (11) in that it has reduced surface abundance of class I molecules that can be increased by incubation of the cells with an appropriate peptide. However, 5.2.4 is unlike 721.174 and T2 in that it retains DP genes from one haplotype, allowing us to examine the function of its class II molecules. We find that deletion mutant 5.2.4 manifests the class II presentation-defective phenotype, and it is noncomplementary with several of the 16.23 selected mutants for the class II presentation defect. Further, the T2 cell line, which is homozygously deleted for a region of the MHC that includes the 5.2.4 deletion, does not complement the mutation in 9.5.3. These findings strongly suggest that defects in a gene(s) mapping within the MHC are responsible for the class II presentation-defective phenotype.

Materials and Methods

APC Lines. With the exception of the T2 cell line, the APC used in these studies are all derived from B-LCL T5-1 (Fig. 1 *a*). The hemizygous deletion mutants 8.1.6 and 9.28.6 were derived directly from T5-1, 8.1.6, by ethyl methane sulfonate (EMS) mutagenesis of T5-1 and selection with an HLA-DR1 antiserum and complement (12), and 9.28.6, by mitomycin *c* mutagenesis and selection with an HLA-B8 antiserum, 0800 (13). The deletion breakpoints in 8.1.6 and 9.28.6 were defined by Southern blot analyses, as described (14). To derive mutant 5.2.4, 8.1.6 was mutagenized with EMS and selected with a monomorphic anti-DR antibody, VI.15, and complement. Presentation-defective mutants 9.5.3, 7.9.6, and 7.2.6 were also isolated from 8.1.6, after EMS mutagenesis and immunoselection with mAb 16.23 plus complement (see reference 5 for details). 3.1.3 is a DR-null cell line derived from 3.1.0, a hemizygous MHC-deletion mutant, which lacks the entire MHC of the DQ2/DR3 haplotype (14). 3.1.3 expresses DQ1 and DP4.1. 9.22.3 is a DR-null cell line, derived from 8.1.6 by deletion of the single DRA gene in 8.1.6 (5). Mutant 721.174, derived from B-LCL 721, bears a homozygous deletion that extends from telomeric to the DPA2 locus to between DRA and the complement gene cluster (8). T1 is a somatic cell hybrid of 721.174 and the T cell line, CEM. A derivative of T1, (T2) was selected for loss of both copies of human chromosome 6 provided by CEM (9). T2 cells expressing transfected DRA and DRB1*0408 cDNAs (T2-DR) were provided by W. Kwok. The cDNAs were introduced in retroviral vectors, as described (15).

T Cells and T Cell Proliferation Assays. The generation of the hepatitis B surface antigen (HBsAg)-specific T cell clone and identification of a stimulatory oligopeptide S1a (amino acid position 14–33) have been described (6). The polyclonal T cell line specific for tetanus toxoid (TT) was generated as described (16). The immunogenic peptide derived from tetanus toxin (amino acid position 947–969) has been described (17). It was found to be restricted by DP4.1 in T5-1 and its derivatives by analysis with class II molecule-loss mutants of T5-1 (18). For assay, 2×10^4 T cell blasts were cocultured with $1–2 \times 10^5$ mitomycin-*c*–treated B-LCLs as APCs in the presence or absence of either native antigen or immunogenic peptide. T cell stimulation was measured by incorporation of [^{3}H]thymidine into DNA (cpm in the presence of antigen − cpm in the absence of antigen). Recombinant HBsAg, the gift of Merck, Sharp, and Dohme (West Point, PA), was used at 10 μg/ml; synthetic HBsAg peptide S1a was used at 1 μg/ml or at varied concentrations, as indicated. Purified TT (Massachusetts Department of Public Health, Jamaica Plain, MA) was used at 5 μg/ml; TT peptide was used at 10 μg/ml.

Somatic Cell Hybrids. For somatic cell hybridization, dominant selectable markers were introduced into 9.5.3, 7.9.6, 7.2.6, and 5.2.4. An *Escherichia coli* gpt gene, conferring resistance to mycophenolic acid, was transfected into 9.5.3, 7.9.6, and 7.2.6 by electroporation. The dihydrofolate reductase gene, conferring resistance to methotrexate, was introduced into 5.2.4, 7.9.6, and 7.2.6 by coculture with PA317-SDHT, a retrovirus-packaging fibroblast line, provided by A. Dusty Miller (19). T2-DR cells containing the hygromycin *b* phosphotransferase gene were kindly provided by W. Kwok (Virginia Mason Research Center, Seattle, WA). Somatic cell hybrids were prepared by polyethylene glycol (PEG) fusion, as described (6). Each hybrid contains both selectable markers by Southern blot analysis (not shown). The tetraploid status of selected hybrids was also confirmed by DNA content determination using propidium iodide staining, FACScan® (Becton Dickinson Immunocytometry Sys., Mountain View, CA) analysis, and CELLFIT® software (not shown).

Immunofluorescent Flow Cytometry. Immunofluorescent analysis was carried out using the following fluoresceinated antibodies: mAb 16.23, anti-DR3 (20); mAb VI.15, anti-DR dimer (13); or unlabeled primary antibodies: mAb ME-1, anti-HLA-B27 (21); mAb PA2.1, anti-HLA A2 (22), followed by fluoresceinated goat F(ab)$_2$ anti–mouse IgG (H+L) (Tago, Inc., Burlingame, CA). In all assays, binding of FITC-labeled goat anti–mouse antibody (GAM) alone was used as control for autofluorescence. Binding to class I–negative Daudi cells (23) was used as a negative control, when appropriate. For antibody binding, cells were incubated with saturating amounts of FITC-conjugated antibody, or unlabeled antibody followed by FITC-labeled GAM. Labeled cells were washed and analyzed on a FACScan® (Becton Dickinson & Co.). Cell number is displayed against a 4 log unit axis of fluorescence intensity (17 channels equals a doubling of intensity).

Induction of HLA-B27 Cell Surface Expression. Cells (3×10^5/ml) were exposed to medium (RPMI 1640 plus 10% FCS) plus DMSO (0.5%) alone or medium plus DMSO and either the HLA-B27-restricted peptide of the HIV-1 gag protein (amino acids 265–279) (24) or S1a, the HLA-DP4-restricted peptide of HBsAg (amino acids 14–33), as a negative control. Both peptides were used at a concentration of 50 μg/ml. Incubation was for 48 h at 37°C. 5×10^5 cells were harvested and stained by indirect immunofluorescence with mAb ME-1, followed by FITC-labeled GAM. Samples were analyzed on a FACScan® (Becton Dickinson & Co.).

Dimer Stability Assay. This assay has been described (5). Briefly, unboiled, whole-cell NP-40 extracts were analyzed by one-dimensional SDS-PAGE and immunoblotted with monoclonal HB10.A, which reacts with DRβ chains as monomers and as

DRα/β dimers (25). The stability of HLA class II dimers to SDS without boiling or reduction has been described (26).

Southern Blot Analyses. These analyses were performed as described (14).

Results

Mutant 5.2.4 Has a Homozygous Deletion within the Class II Region of the MHC. The progenitor of both the newly isolated mutant 5.2.4 and of the 16.23 selected mutants (e.g., 9.5.3) was 8.1.6, which in turn was isolated from the B lymphoblastoid line T5-1 (Fig. 1 *a*). 8.1.6 has a hemizygous deletion of its MHC DQ1/DR1 haplotype whose breakpoints map telomeric of the DP genes and centromeric of the C4 genes (14). More precise mapping of the deletion breakpoints (Fig. 1 *b*) demonstrates that the X1–6 genes located immediately centromeric of the 21-OHB gene, and the Y4 and Y5 genes located telomeric of the DNA gene, lie outside the 8.1.6 deletion and thus delimit its centromeric and telomeric breakpoints (27). Mutant 5.2.4 has undergone a second deletion, in MHC haplotype DQ2/DR3. From serological analyses (data not shown) the second deletion appears to have removed the entire MHC of the DQ2/DR3 haplotype. This was confirmed by Southern blot analyses, which indicate that 5.2.4 has a homozygous deletion of the DR/DQ region and a hemizygous deletion of DP and class I genes (Fig. 1 *c*).

Class II-restricted Antigen Presentation Is Impaired in Mutant 5.2.4. To evaluate the ability of 5.2.4 to present class II–restricted antigens to T cells, we studied its ability to present HBsAg to a specific, DP4-restricted T cell clone. Soluble HBsAg must be processed to yield the epitope recognized by these T cells and a synthetic peptide corresponding to amino acids 14–33 of HBsAg (S1a) is also stimulatory for this clone (6). We compared presentation of HBsAg by 5.2.4 to that of its progenitor, 8.1.6, and to a DP-hemizygous cell line, 9.28.6 (see Fig. 1 *a*), the latter to control for any effect of the DP gene dosage difference between 8.1.6 (dizygous) and 5.2.4 (hemizygous). The progenitor line, 8.1.6, and the 9.28.6 control line effectively present both native HBsAg and the synthetic peptide corresponding to the HBsAg epitope (Fig. 2 *a*). In contrast, mutant 5.2.4 is defective for presentation of the native antigen, but effectively presents the HBsAg peptide. Likewise, presentation of native TT is defective in 5.2.4, whereas a DP-restricted TT peptide is presented normally (Fig. 2 *a*). These results indicate that mutant 5.2.4 has a defect resembling that found in the previously characterized 16.23-selected mutants in that it is defective for presentation of intact protein antigens to class II–restricted T cells, but effectively presents the peptide epitope from those antigens.

Both 16.23 Selected Mutants and 5.2.4 Have an Enhanced Capacity to Present Peptide Antigen. We have hypothesized that the altered conformation of cell surface class II molecules in the 16.23-selected mutants reflects reduced occupancy of their peptide-binding grooves (6). To evaluate the possibility that the binding grooves of the DP molecules expressed by 5.2.4 are empty or under-occupied, we compared the ability of 5.2.4 and a control DP hemizygous line, 9.28.6, to stimulate a specific T cell response over a range of concentrations of peptide antigen. Mutant 5.2.4 shows an ~15-fold enhancement in HBsAg peptide presentation compared with 9.28.6 (Fig. 2 *b*). Presentation by 5.2.4 of another DP-restricted peptide, amino acids 947–969 of TT, is also enhanced to a similar degree (T. Monji, data not shown). Mutants 9.5.3, 7.9.6, and 7.2.6, representative of the 16.23-selected mutants, also show enhanced presentation of the DP-restricted HBsAg peptide, compared with progenitor 8.1.6 (Fig. 2 *c*). Thus, both 5.2.4 and the set of mutants represented by 9.5.3 present DP-restricted peptides more effectively than wild-type cells. These results are consistent with the hypothesis that these mutants bind class II–restricted peptides more avidly than the progenitor because the binding grooves of their class II dimers are empty or underoccupied with self-peptides.

MHC Deletion Mutant 5.2.4 Is Noncomplementary with the 16.23 Selected Mutant 9.5.3. To evaluate the possibility that the gene responsible for the class II presentation defect in the homozygous deletion mutant 5.2.4 is the same as that altered in mutant 9.5.3, we made a somatic cell hybrid between 5.2.4 and 9.5.3 and assayed the hybrid for complementation of the mutant phenotype. To assess complementation, we examined the conformational state of the DR molecules expressed by the 5.2.4 × 9.5.3 hybrid. In two independent hybrids, the DR molecules retain the mutant phenotype: the hybrids fail to bind the 16.23 antibody (Fig. 3 *a*), and extracted DR dimers from the hybrids dissociate into monomers in SDS-PAGE (Fig. 3 *b*). Thus, the conformational defect in the DR molecules expressed by 9.5.3 is not restored by hybridization with 5.2.4. These hybrid cells are also impaired for presentation of intact TT, but effectively present a DP-restricted peptide derived from TT (Fig. 3 *c*). By these three criteria, therefore, the hybrids made between 9.5.3 and 5.2.4 maintain the mutant phenotype. Further, as the lesions in both 9.5.3 and 5.2.4 act as recessives in somatic cell hybrids (see Table 1), the mutant phenotype of the 9.5.3 × 5.2.4 hybrid indicates that the mutations affecting class II molecules in 9.5.3 and 5.2.4 are noncomplementary. Thus, it appears that the same gene is defective in both cells, making it likely that the relevant gene is MHC linked.

T2, a Second Homozygous MHC Deletion Mutant, Is Also Noncomplementary with 9.5.3. It was formally possible that a second mutation in 5.2.4, in addition to its MHC deletion, was responsible for the class II presentation defect in mutant 5.2.4 and for its lack of complementation with mutant 9.5.3. To address this possibility, we utilized T2, a class II–negative, MHC deletion mutant not related to 5.2.4, but with a slightly larger homozygous deletion that includes that of 5.2.4 (references 9, 27). We first asked whether class II molecules introduced into T2 by transfection manifest the mutant phenotype. This proved to be the case; DR molecules in a T2-DR transfectant are expressed stably as dimers at the cell surface (Fig. 3 *a*) but dissociate into monomers in SDS-PAGE (Table 1). Second, we made a somatic cell hybrid between mutant 9.5.3 and the T2-DR transfectant. The DR3 molecules expressed by this hybrid retain the mutant phenotype, as they fail to regain binding of the 16.23 mAb (Fig.

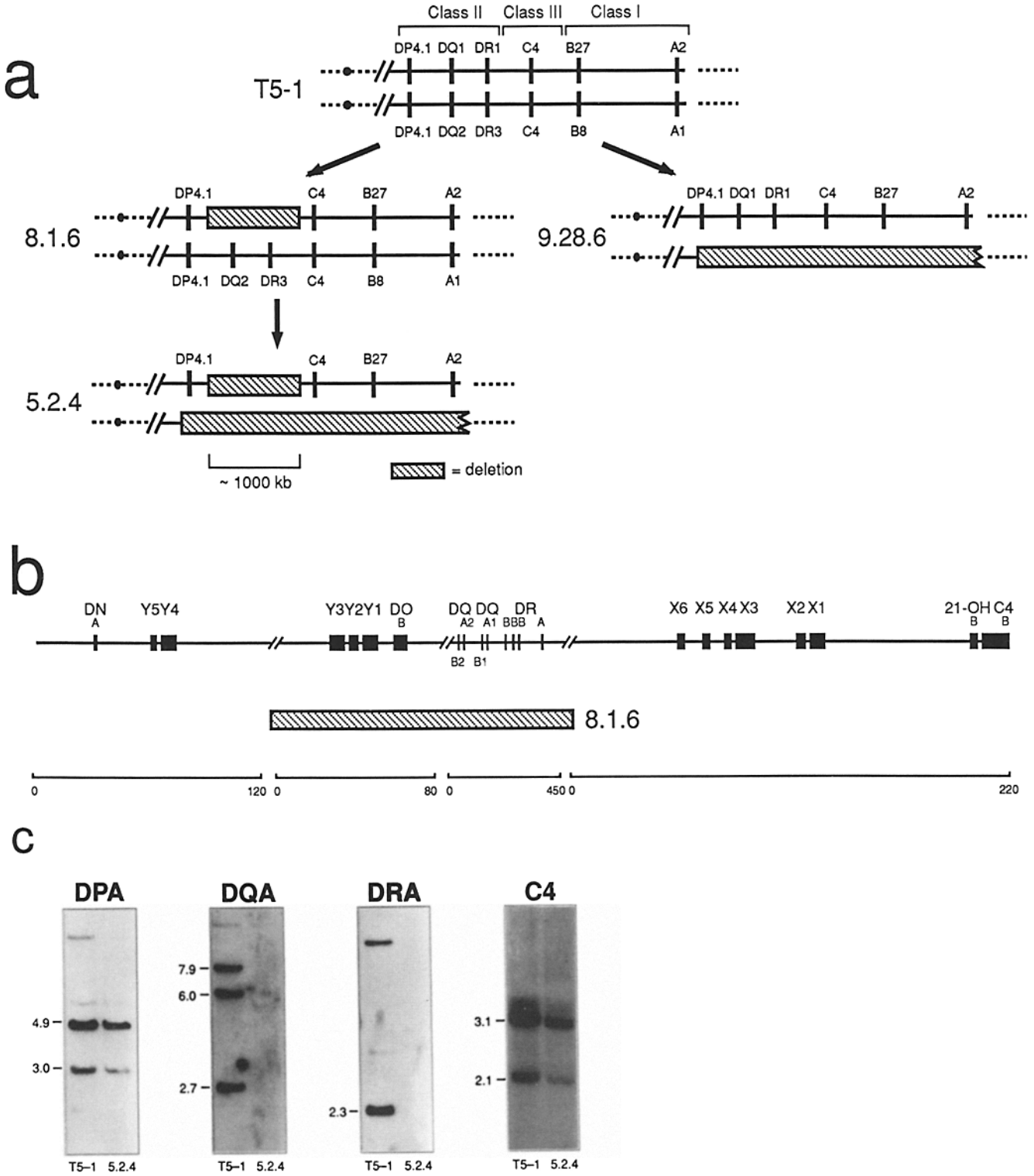

Figure 1. (*a*) Derivation of mutants 8.1.6, 9.28.6, and 5.2.4 from cell line T5-1. Extended MHC haplotypes are shown. The vertical bars indicate the approximate chromosomal locations of the genetic loci (e.g., *DRA* and *DRB*) encoding each of the expressed proteins. (*b*) Genetic map of the relevant portion of the MHC class II and class III regions with delineation of 8.1.6 deletion breakpoints. Scale in kb. (Adapted from (reference 27) with permission copyright (*c*), 1990 Macmillan Magazines Ltd.). (*c*) Mutant 5.2.4 has a deletion within the class II region of the MHC. HindIII-digested DNA from 5.2.4 and progenitor T5-1 (see Fig. 1 *a*) was analyzed by Southern blotting, using the following cDNA probes: DPB1 (31, 32); DPA1 (33); DQA1 (34); DQB1 (34); DRB3 (35); DRA (36); C4 (37); and HLA 1.3, an HLA-B7 class I gene (38). Representative blots, which define the extent of the homozygous deletion in mutant 5.2.4, are shown. (*DPA*) Southern blots of T5-1 DNA probed with DPA cDNA have two major bands and two weak bands, all of which are reduced in intensity (~50%) in 5.2.4. These findings indicate the loss of DPA genes of one haplotype. (DQA) The DQA1 cDNA clone hybridized with two DQA-like genes (34) which are represented by 7.9-, 6.0-, and 2.7-kb bands in T5-1 DNA. The 7.9-

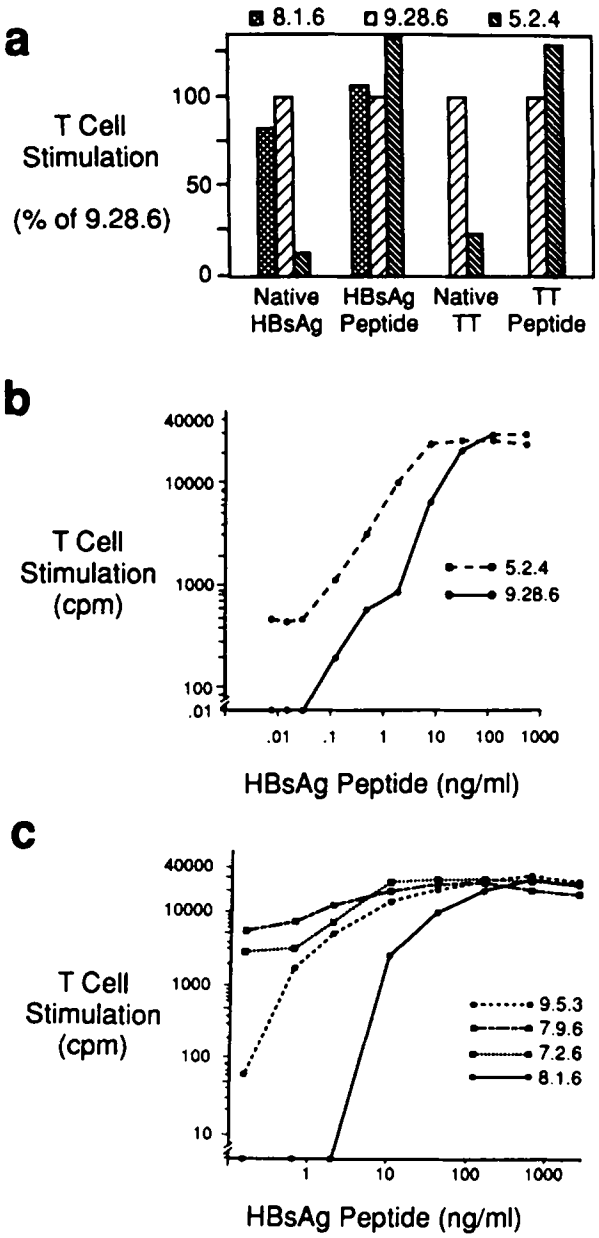

Figure 2. Antigen presentation by DP molecules is altered in mutant 5.2.4. (*a*) Proliferation of antigen-specific T cells stimulated with native antigen or immunogenic peptide, presented by progenitor 8.1.6, DP-hemizygous line, 9.28.6 (see Fig. 1 *a*), and mutant 5.2.4. T cells are DP4-restricted and specific for HBsAg or TT. T cell stimulation was measured by [^{3}H]thymidine incorporation as described (16). Data are expressed as percent of stimulation by 9.28.6 and are median values from triplicate cultures of a representative experiment; SEM <15% in at least three experiments. Stimulation of HBsAg-specific T cells by 9.28.6 with intact protein, 29,147 cpm; with peptide, 63,654 cpm; stimulation of TT-specific T cells by 9.28.6 with intact protein, 9,305 cpm, with peptide, 9,063 cpm. The modest T cell stimulation in response to 5.2.4 cells exposed to intact antigen may be due to the fact that the 5.2.4 mutation is "leaky," i.e., some class II/peptide complexes form in 5.2.4, despite deletion of the relevant gene. Alternatively, the whole antigen preparations may contain small amounts of denatured or fragmented antigen which can be presented without further processing. For the polyclonal TT-specific T cell line, a third possibility is that the line includes a subpopulation of T cells responsive to complexes generated in a pathway which is unaltered in 5.2.4. The response of this line is similar in magnitude to the response of other polyclonal T cell lines to the 16.23 mutants (6). (*b*) Proliferation of HBsAg-specific T cells stimulated with HBsAg peptide at indicated concentrations, using 5.2.4 or 9.28.6 as APCs. T cell stimulation was calculated as in (*a*). (*c*) Proliferation of HBsAg-specific T cells stimulated with HBsAg peptide at indicated concentrations, using representative presentation-defective mutants, 9.5.3, 7.9.6, 7.2.6, or progenitor 8.1.6 as APCs. T cell stimulation was calculated as in (*a*).

3 *a*). These findings confirm the conclusion that a gene responsible for the class II presentation-defective phenotype maps within the one-megabase MHC region deleted in mutant 5.2.4, and, *pari passu*, within the larger deletion in T2.

The Defects in Mutant 9.5.3, 7.2.6, and 7.9.6 Are Noncomplementary. The phenotype of the 16.23-selected mutants might result from lesions in different genes, each of which is required for the intracellular formation of class II molecule–peptide complexes. To investigate this, we made a series of somatic cell hybrids between independently arising 16.23-selected mutants (5, 6). To assess complementation, we measured binding of the 16.23 antibody and the stability of DR dimers in SDS-PAGE. We first determined that the genetic lesions in mutants 7.9.6 and 7.2.6 are recessive. Somatic cell hybrids made between these mutants and nonmutant B-LCLs have stable DR dimers and bind mAb 16.23 (Table 1). The mutation in 9.5.3 is also recessive (Table 1 and reference 6). The 9.5.3 × 7.2.6 hybrid, the 9.5.3 × 7.9.6 hybrid, and the 7.2.6 × 7.9.6 hybrid all retain the mutant phenotype for DR dimer stability (Table 1), suggesting that these mutants constitute a single complementation group. None of these hybrids have regained normal binding of mAb 16.23 (Table 1). It thus appears that a single, MHC-linked gene is responsible for the class II presentation-defective phenotype in mutants 9.5.3, 7.9.6, and 7.2.6.

Cell Surface Expression of Class I MHC Molecules Is Reduced in Mutant 5.2.4, but Not in 16.23 Selected Mutants. An MHC-linked gene, peptide supply Factor 1 (PSF1), is implicated in the control of cell surface abundance of class I MHC molecules (27, 28). In mutant cells that lack mRNA for this

and 6.0-kb bands represent DQA1 alleles and the 2.7-kb band represents a DQA1-like sequence, DQA2 (14) which maps centromeric of both DQA1 and DQB1. All bands are missing in 5.2.4, indicating homozygous deletion of DQA1 and DQA2. (*DRA*) T5-1 DNA digested with HindIII and probed with DRA yields two bands. Both bands are absent in mutant 5.2.4, indicating homozygous deletion of DRA genes. (*C4*) The fourth component of the human complement system: the two bands detected by the C4 cDNA in T5-1 DNA are reduced in intensity and therefore deleted on one haplotype in mutant 5.2.4. The blots were also analyzed with the HLA-1.3 probe, which cross-hybridizes with a large number of class I sequences. There is a reduction in intensity of ~50% in the class I bands from mutants 5.2.4 compared with T5-1, and a 5.8-kb band, which represents a class I sequence of the DR3 haplotype (14), is missing in 5.2.4 DNA (not shown). These findings indicate deletion of class I sequences of the DR3 haplotype in mutant 5.2.4. (*Ii* and B_2m) Blots were also analyzed with probes for these genes which are unlinked to the MHC, as controls for amount of DNA loaded. No differences between T5-1 and 5.2.4 DNA were observed (not shown).

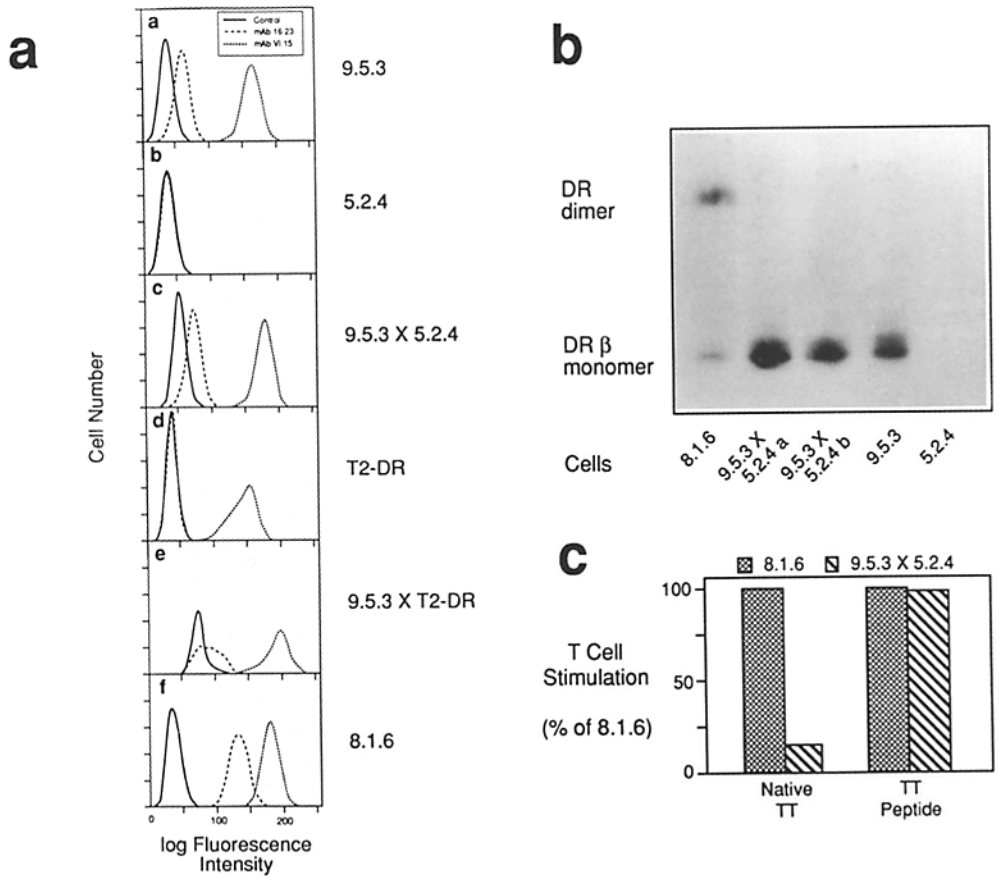

Figure 3. Somatic cell hybrids made between 9.5.3 and 5.2.4, and between 9.5.3 and T2 express the mutant phenotype. (*a*) A 9.5.3 × 5.2.4 hybrid binds mAb 16.23 at levels equivalent to mutant 9.5.3; a 9.5.3 × T2-DR hybrid binds the 16.23 mAb at levels equivalent to 9.5.3 as well. Immunofluorescent analysis was carried out using the following fluoresceinated antibodies and cells. Control (_____), goat anti-mouse antibody; 16.23, anti-DR3, (----): (reference 20); VI.15, anti-DR dimer (...):(reference 13). All the curves for mutant 5.2.4 (*b*) are superimposed, as 5.2.4 expresses no cell surface DR. For T2-DR (*d*), the mAb 16.23 curve is superimposed on the control antibody curve. The T2-DR transfectant does not bind the DR3-specific mAb 16.23, because the introduced β chain expresses the DR4 specificity. The failure of the 9.5.3 × T2-DR hybrid to express the 16.23 epitope is not due to segregation of the chromosome 6 encoding the DR3 B1 gene, because the hybrid expresses the HLA-B5 molecule (not shown) and HLA-B5 surface expression requires a functional PSF1 gene (28), which must be contributed by the (DR3+) chromosome 6 from mutant 9.5.3. (*b*) The HLA-DR dimers extracted from 9.5.3 and two independent hybrids (9.5.3 × 5.2.4a, 9.5.3 × 5.2.4b) dissociate into monomeric subunits in one-dimensional SDS-PAGE. A Western blot analysis of detergent (NP-40) extracts of indicated cell lines, using mAb HB10A, is shown. (*c*) Proliferation of TT-specific T cells stimulated with native TT or TT peptide (947–969) presented by progenitor 8.1.6 and by the 9.5.3 × 5.2.4 hybrid. T cell stimulation was measured by [^{3}H]thymidine incorporation. Data are expressed as percent of stimulation by 8.1.6 and are median values from triplicate cultures of a representative experiment (SEM <15%). Similar results were obtained in three experiments. Stimulation of TT-specific T cells by 8.1.6 with native TT, 10,534 cpm;; with peptide 7,093 cpm.

gene, assembly and cell surface expression of class I molecules are defective, but can be restored by incubation with peptides that bind to the class I molecules expressed by the cell (27). Mutant 5.2.4, which is homozygously deleted for PSF1, shares this phenotype. Its surface level of HLA-A2 is reduced by ~50% (Fig. 4 *a*) and that of HLA-B27 is reduced by ~85% compared to progenitor 8.1.6 (Fig. 4 *b*). Incubation of 5.2.4 with peptides that bind to HLA-B27, HIV gag 265–279 (Fig. 4 *d*), or influenza nucleoprotein 383–394 (not shown) enhances HLA-B27 cell surface expression two fold, whereas incubation with a DP-restricted peptide has no effect (Fig. 4 *c*). In contrast, PSF1 function does not appear to be defective in mutants 9.5.3, 7.2.6, and 7.9.6, as evidenced by their normal cell surface levels of HLA-A2 and B27 (Fig. 4, *a* and *b*). Mutant 5.2.4 thus differs from the 16.23-selected mutants in the formation of intracellular complexes between class I molecules and peptides, suggesting that lesions in different genes underlie the class I- and class II-defective phenotypes in mutant 5.2.4.

Discussion

In a previous study, we characterized a set of mutants of an EB virus–transformed B cell line that are defective in their ability to function as class II–restricted antigen-presenting cells when exposed to intact exogenous antigens (16.23-selected mutants). At that time, the genetic basis for the defect was not determined. In the present study, we have explored the genetics of the class II presentation defect using a homozygous MHC deletion mutant, 5.2.4. We were first led to investigate class II–restricted presentation in 5.2.4 because the 16.23-selected mutants arose at a high frequency ($\sim 10^{-4}$) in mutagenized cell line 8.1.6, which has a hemizygous MHC deletion (see Fig. 1 *a*). The fact that no such mutants could be isolated from comparably mutagenized T5-1, the undeleted progenitor of 8.1.6, (E. Mellins and D. Pious, unpublished results) implicated the region of the MHC which is hemizygous in 8.1.6 and homozygously deleted in 5.2.4 as a possible locus of the involved gene.

The phenotypic changes associated with the presentation

Table 1. *Summary of DR Dimer Stability and mAb 16.23 Binding Assays in T2-DR Transfectant and in (Mutant × Wild Type) and (Mutant × Mutant) Hybrids*

	Dimer stability	16.23 binding
T2-DR transfectant	–	0*
Hybrids		
(Mutant × wild type)		
9.5.3 × 3.1.3	+	+
5.2.4 × 8.1.6	ND	+
7.9.6 × 3.1.3	+	+
7.2.6 × 9.22.3	+	+
(Mutant × mutant)		
5.2.4 × 7.9.6	–	–
5.2.4 × 7.2.6	–	–
9.5.3 × 7.9.6	–	–
9.5.3 × 7.2.6	–	–
7.9.6 × 7.2.6	–	–

Dimer stability was determined in Western blot analyses, as in Fig. 3 B. Binding of mAb 16.23 was determined by FACS® analyses, as in Fig. 3 A. For 16.23 binding, + signifies binding at >80% of levels bound by 8.1.6, normalized for level of cell surface DR; – signifies binding at levels equivalent to 16.23-selected mutants, which range from 0 to 20% of 8.1.6 levels. Cell lines 3.1.3 and 9.22.3 are both DR-null B-LCLs, which present DQ- and DP-restricted antigens normally (see Materials and Methods). Data on the 9.5.3 × 3.1.3 hybrid have been previously reported (6).

* T2-DR expresses the DR4 specificity only and fails to bind the anti-DR3 mAb 16.23, as expected.

defect in the 16.23-selected mutants, and the fact that 5.2.4 retains expressed DPA and DPB genes, provided the means to assess this function in 5.2.4. Although 5.2.4 expresses DP molecules on the cell surface, it is defective in the presentation of DP-restricted intact protein antigens to antigen-specific T cells. It presents DP-restricted antigenic peptides efficiently, however. In fact, in this study we show that both 5.2.4 and the previously isolated mutants present peptides more efficiently than does the progenitor. The enhanced presentation of peptides is consistent with the interpretation that class II molecules on the cell surface of these mutants are un- or underoccupied with self-peptides. The fact that 5.2.4 had acquired the class II presentation defect which characterizes the previously isolated mutants coincidently with a homozygous MHC deletion further implicated the deleted region as the site of the involved gene.

Given the phenotypic similarities of the defects in 5.2.4 and the 16.23-selected mutants, it became of interest to determine whether defects in the same or different genes underlie the presentation abnormalities. The result is clear: 5.2.4 fails to complement three independently arising 16.23-selected mutants, and the latter fail to complement each other. This is consistent with the interpretation that all four mutants are defective in the same gene(s). Based on the complementation results and the association of the class II presentation defect in 5.2.4 with the homozygous deletion in the class II region, we tentatively concluded that the affected gene in all four mutants maps to the deleted region. However, it remained possible, although unlikely, that 5.2.4 could have an occult second genetic lesion elsewhere in the genome that was responsible for its presentation defect, and that this gene was also defective in the 16-23-selected mutants. If this were the case, a second, independently arising mutant with a homozygous deletion that includes the MHC deletion in 5.2.4 should complement the class II presentation defect in the 16.23-selected mutants. T2 has a homozygous MHC deletion that includes that of 5.2.4. However, T2 failed to rescue the mutant phenotype in a hybrid between T2 and mutant 9.5.3, again consistent with the mapping of the presentation defect to the deletion. In addition, DR dimers expressed by a T2-DR transfectant demonstrate the in vitro instability characteristic of the class II mutant phenotype (Table 1), and DR3 dimers expressed by a T2-DR3 transfectant fail to bind the 16.23 antibody (P. Cresswell, personal communication). For these reasons we conclude that a gene(s) whose defect is responsible for the class II presentation abnormality in 5.2.4 and the 16.23-selected mutants maps to the MHC class II region.

Within the 5.2.4 deletion implicated in the class II presentation defect, the only known genes, besides class II structural genes, are a cluster of genes mapping between DNA and DOB (Fig. 1 *b* and reference 27). Two of these, designated Y1 and Y3, are homologous to bacterial transport and mammalian multi-drug resistance genes. The Y3 gene product, PSF1, is required for normal cell surface abundance of class I molecules. A mutant, 721.134, which lacks Y3 mRNA, has reduced surface levels of class I molecules and is complemented for this defect by transfection of an expressible Y3 gene (27, 28). However, a similar defect in class I molecule expression in the MHC deletion mutant 721.174 is not restored by transfection of Y3, suggesting that another gene(s) in this region is also required for normal cell surface expression of class I molecules (28). Thus, the deletion of Y3, and possibly of this other unidentified gene(s), is likely to explain the lowered cell surface level of class I molecules in mutant 5.2.4.

Deletion of Y3 seems unlikely to explain the class II presentation defect in 5.2.4, however. The 16.23-selected mutants 9.5.3, 7.9.6, and 7.2.6 are noncomplementary with 5.2.4 for the class II presentation defect, but they have normal surface abundance of class I molecules, indicating that their Y3 genes are functioning normally. Abnormalities in the Y1 gene, which, like Y3, is homologous to transport genes, could account for the defect in class II–restricted antigen presentation in 5.2.4 and the 16.23-selected mutants. If the class II presentation defect in these mutants were caused by defects in Y1, it would suggest that class II–restricted peptides must be transported across a membrane. Other candidate genes that might be defective in the class II presentation mutants include two MHC-linked genes encoding the polymorphic subunits of an intracellular complex, low molecular weight polypeptide (LMP) (29). The biological function of the LMP complex is unknown, but it shares some structural features

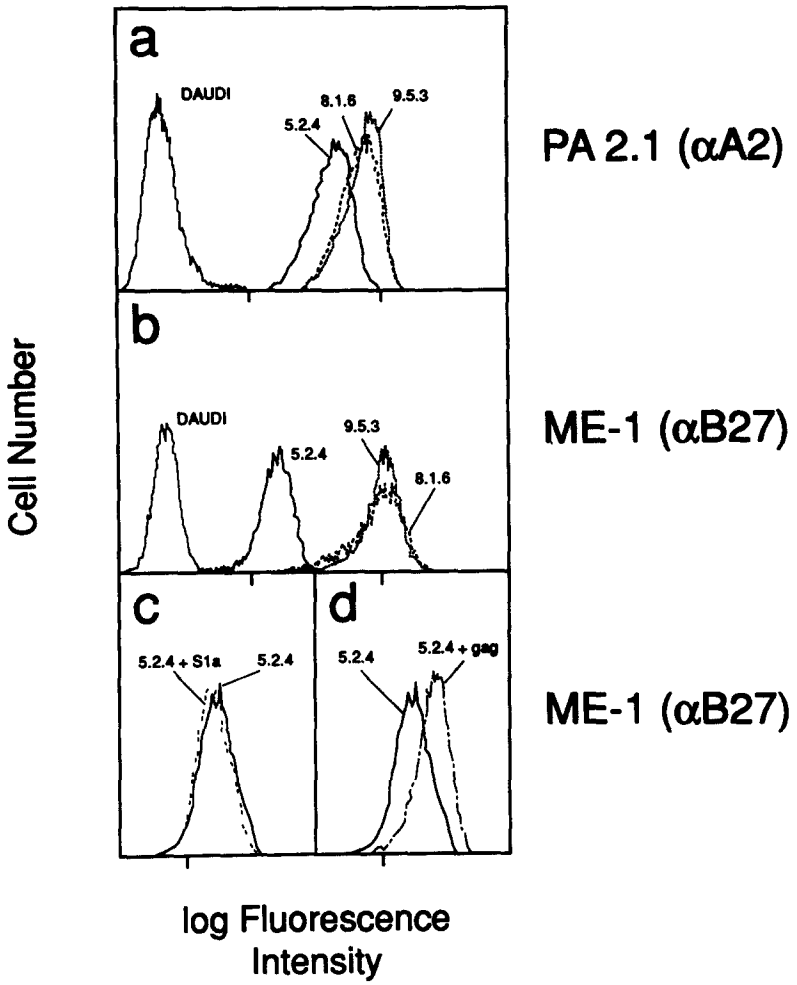

Figure 4. Cell surface expression of class I MHC molecules is reduced on 5.2.4, but not on 9.5.3, and can be specifically induced on 5.2.4 by a class I-restricted peptide. (*a*) Mutants 5.2.4 and 9.5.3 and progenitor line 8.1.6 were stained with HLA-A2-specific antibody PA2.1 by indirect immunofluorescence. Staining of class I-negative cell line, Daudi, is shown as negative control. 16.23-selected mutants, 7.2.6 and 7.9.6, bind mAb PA2.1 at levels equivalent to that of mutant 9.5.3 (not shown). (*b*) Mutants 5.2.4 and 9.5.3 and progenitor line 8.1.6 were stained with HLA-B27-specific antibody ME-1 by indirect immunofluorescence. Mutants 7.2.6 and 7.9.6 bind mAb ME-1 at levels equivalent to that of mutant 9.5.3 (not shown). (*c*) 5.2.4 cells stained with ME-1 after incubation with HBsAg peptide S1a. HBsAg peptide S1a was used at 2.3×10^{-5} M and at 3.5×10^{-5} M, with equivalent results. (*d*) 5.2.4 cells stained with ME-1 after incubation with HIV gag peptide. HIV gag peptide was used at 2.8×10^{-5} M. An equivalent induction of ME-1 binding to that observed with the gag peptide was observed after 5.2.4 incubation with another HLA-B27-restricted peptide, NP1, derived from residues 383–394 of the influenza nucleoprotein (not shown). No increase in ME-1 binding to progenitor 8.1.6 was observed after incubation with HLA-B27-restricted peptides (not shown).

of the proteolytic proteosome complex (30). A final possibility is that the relevant gene is an as yet unrecognized gene mapping to the region encompassed by the 5.2.4 deletion.

Our study has demonstrated for the first time that genes within the MHC other than the classical MHC structural genes are required for class II-restricted antigen presentation. These results, taken together with the recent finding of an MHC-linked function required for class I-restricted presentation, yield an emerging picture of striking conservation of MHC linkage of genes involved in antigen presentation.

We thank Merck, Sharp and Dohme for the gift of purified HBsAg; J. Goldman, who initially isolated mutant 5.2.4 while in our lab; Drs. Andrew MacMichael, Edward Clark, and Peter Parham for antibodies; Drs. William Kwok and Peter Cresswell for the T2 cell line; and Dan Hill for preparation of the manuscript.

This work was supported by National Institutes of Health grants AI-28809 (E. Mellins); and HG-00243 and AI-30527 (D. Pious).

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Received for publication 28 June 1991 and in revised form 20 August 1991.

References

1. Yewdell, J.W., and J.R. Bennick. 1990. The binary logic of antigen processing and presentation to T cells. *Cell.* 60:203.
2. Buus, S., A. Sette, S.M. Colon, and H.M. Grey. 1988. Autologous peptides constitutively occupy the antigen binding site on Ia. *Science (Wash. DC).* 242:1045.
3. Demotz, S., H.M. Grey, and A. Sette. 1990. The minimal

number of class II MHC-antigen complexes needed for T cell activation. *Science (Wash. DC).* 249:1028.
4. Harding, C.V., and E.R. Unanue. 1990. Quantitation of antigen-presenting cell MHC class II/peptide complexes necessary for T-cell stimulation. *Nature (Lond.).* 346:574.
5. Pious, D., L. Dixon, F. Levine, T. Cotner, and R. Johnson. 1985. HLA class II regulation and structure: analysis with HLA-DR3 and HLA-DP point mutants. *J. Exp. Med.* 162:1193.
6. Mellins, E., L. Smith, B. Arp, T. Cotner, E. Celis, and D. Pious. 1990. Defective processing and presentation of exogenous antigens in mutants with normal HLA class II genes. *Nature (Lond.).* 343:71.
7. Krieg, D.R. 1963. Ethyl methanesulfonate-induced reversion of bacteriophage T4rII mutants. *Genetics.* 48:561.
8. DeMars, R., R. Rudersdorf, C. Chang, J. Petersen, J. Strandtmann, N. Korn, B. Sidwell, and H.T. Orr. 1985. Mutations that impair a posttranscriptional step in expression of HLA-A, and -B antigens. *Proc. Natl. Acad. Sci. USA.* 82:8183.
9. Salter, R.D., D.N. Howell, and P. Cresswell. 1985. Genes regulating HLA class I antigen expression in T-B lymphoblast hybrids. *Immunogenetics.* 21:235.
10. Cerundolo, V., J. Alexander, K. Anderson, C. Lamb, P. Cresswell, A. McMichael, F. Gotch, and A. Townsend. 1990. Presentation of viral antigen controlled by a gene in the major histocompatibility complex. *Nature (Lond.).* 345:449.
11. Townsend, A., Öhlén, J. Bastin, H.-G. Ljunggren, L. Foster, and K. Kärre. 1989. Association of class I major histocompatibility heavy and light chains induced by viral peptides. *Nature (Lond.).* 340:443.
12. Gladstone, P., and D. Pious. 1978. Stable variants affecting B cell alloantigens in human lymphoid cells. *Nature (Lond.).* 271:459.
13. Gladstone, P., L. Fueresz, and D. Pious. 1982. Gene dosage and gene expression in the HLA region: evidence from deletion variants. *Proc. Natl. Acad. Sci. USA.* 79:1235.
14. Levine, F., H. Erlich, B. Mach, R. Leach, R. White, and D. Pious. 1985. Deletion mapping of HLA and chromosome 6p genes. *Proc. Natl. Acad. Sci. USA.* 82:3741.
15. Kwok, W.W., D. Schwarz, B. Nepom, P. Thurtle, R. Hock, and G.T. Nepom. 1988. HLA-DQ molecules from α-β heterodimers of mixed allotype. *J. Immunol.* 141:3123.
16. Mellins, E., M. Woelfel, and D. Pious. 1987. Importance of HLA-DQ and -DP restriction elements in T-cell responses to soluble antigens: mutational analysis. *Hum. Immunol.* 18:211.
17. Demotz, S., P.M. Matricardi, C. Irle, P. Panina, A. Lanzavecchia, and G. Corradin. 1989. Processing of tetanus toxin by human antigen-presenting cells. Evidence for donor and epitope-specific processing pathways. *J. Immunol.* 143:3881.
18. Monji, T., E. Mellins, T. Cotner, and D. Pious. 1990. Further characterization of tetanus toxin T cell antigenic peptides restricted by the DR3, DQ2 haplotype. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 4:608a. (Abstr.)
19. Miller, A.D., and C. Buttimore. 1986. Redesign of retrovirus packaging cell lines to avoid recombination leading to helper virus production. *Mol. Cell. Biol.* 6:2895.
20. Johnson, J.P., T. Meo, G. Riethmüller, D.J. Schendel, and R. Wank. 1982. Direct demonstration of an HLA-DR allotypic determinant on the low molecular weight (beta) subunit using a mouse monoclonal antibody specific for DR3. *J. Exp. Med.* 156:104.
21. Ellis, S.A., C. Taylor, and A. McMichael. 1982. Recognition of HLA-B27 and related antigen by a monoclonal antibody. *Hum. Immunol.* 5:49.
22. Parham, P., and W.F. Bodmer. 1978. Monoclonal antibody to a human histocompatibility alloantigen, HLA-A2. *Nature (Lond.).* 276:397.
23. Nillson, K., P.-E. Evrin, and K.I. Welsh. 1974. Production of β_2-microglobulin by normal and malignant human cell lines and peripheral lymphocytes. *Transplant. Rev.* 21:53.
24. Nixon, D.F., A.R.M. Townsend, J.G. Elvin, C.R. Rizza, J. Gallwey, and A.J. McMichael. 1988. HIV-1 gag-specific cytotoxic T lymphocytes defined with recombinant vaccinia virus and synthetic peptides. *Nature (Lond.).* 336:484.
25. Clark, E., and T. Takoshi. 1984. *In* Leucocyte Typing. A. Bernard, L. Baunsell, J. Dausset, and S. Schlossman, editors. Springer-Verlag, Heidelberg. 195–202.
26. Billing, R., M. Safani, and P.J. Peterson. 1976. Isolation and characterization of human B cell alloantigens. *J. Immunol.* 117:1589.
27. Spies, T., M. Bresnahan, S. Bahram, D. Arnold, G. Blanck, E. Mellins, D. Pious, and R. DeMars. 1990. A gene in the human major histocompatibility complex class II region controlling the class I antigen presentation pathway. *Nature (Lond.).* 348:744.
28. Spies, T., and R. DeMars. 1991. Restored expression of major histocompatibility class I molecules by gene transfer of a putative peptide transporter. *Nature (Lond.).* 351:323.
29. Monaco, J.J., and H.O. McDevitt. 1984. H-2-linked low-molecular weight polypeptide antigens assemble into an unusual macromolecular complex. *Nature (Lond.).* 309:797.
30. Monaco, J.J., and H.O. McDevitt. 1986. The LMP Antigens: a stable MHC-controlled multisubunit protein complex. *Hum. Immunol.* 15:416.
31. Roux-Dosseto, M., C. Auffray, J.W. Lillie, J.M. Boss, D. Cohen, R. DeMars, C. Mawas, J.G. Seidman, and J.L. Strominger. 1983. Genetic mapping of a human class II antigen β-chain cDNA clone to the SB region of the HLA complex. *Proc. Natl. Acad. Sci. USA.* 80:6036.
32. Long, E.O., J. Gorski, and B. Mach. 1984. Structural relationship of the SB-chain gene to HLA-D-region genes and murine I-region genes. *Nature (Lond.).* 310:233.
33. Gorski, J., P. Rollini, E. Long, and B. Mach. 1984. Molecular organization of the HLA-SB region of the human major histocompatibility complex and evidence for two SB β-chain genes. *Proc. Natl. Acad. Sci. USA.* 81:3934.
34. Auffray, C., A. Ben-Nun, M. Roux-Dosseto, R.N. Germain, J.G. Seidman, and J.L. Strominger. 1983. Polymorphism and complexity of the human DC and murine I-A α chain genes. *EMBO J.* 2:121.
35. Long, E., C. Wake, J. Gorski, and B. Mach. 1983. Complete sequence of an HLA-DR β chain deduced from a cDNA clone, an identification of multiple non-allelic DRβ chain genes. *EMBO (Eur. Mol. Biol. Organ.) J.* 2:389.
36. Stetler, D., H. Das, J.H. Nunberg, R. Saiki, R.S. Dong, K.B. Mullis, S.M. Weissman, and H.A. Erlich. 1982. Isolation of a cDNA clone for the human HLA-DR antigen α chain by using a synthetic oligonucleotide as a hybridization probe. *Proc. Natl. Acad. Sci. USA.* 79:5966.
37. Weitkamp, L.R., and L.U. Lamm. 1982. Report of the committee on the genetic constitution of chromosome 6. *Cytogenet. Cell Genet.* 32:130.
38. Sood, A.K., D. Pereira, and S.M. Weissman. 1981. Isolation and partial nucleotide sequence of a cDNA clone for human histocompatibility antigen HLA-B by use of an oligodeoxynucleotide primer. *Proc. Natl. Acad. Sci. USA.* 78:616.