Novel Mutants Define Genes Required for the Expression of Human Histocompatibility Leukocyte Antigen DM: Evidence for Loci on Human Chromosome 6p

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Summary

We and others have shown that the products of the HLA-DM locus are required for the intracellular assembly of major histocompatibility complex class II molecules with cognate peptides for antigen presentation. HLA-DM heterodimers mediate the dissociation of invariant chain (Ii)-derived class II–associated Ii peptides (CLIP) from class II molecules and facilitate the loading of class II molecules with antigenic peptides. Here we describe novel APC mutants with defects in the formation of class II–peptide complexes. These mutants express class II molecules which are conformationally altered, and an aberrantly high percentage of these class II molecules are associated with Ii-derived CLIP. This phenotype resembles that of DM null mutants. However, we show that the defects in two of these new mutants do not map to the DM locus. Nevertheless, our evidence suggests that the antigen processing defective phenotype in these mutants results from deficient DM expression. These mutants thus appear to define genes in which mutations have differential effects on the expression of conventional class II molecules and DM molecules. Our data are most consistent with these factors mapping to human chromosome 6p. Previous data have suggested that the expression of DM and class II genes are coordinately regulated. The results reported here suggest that DM and class II can also be differentially regulated, and that this differential regulation has significant effects on class II–restricted antigen processing.

Conventional class II molecules of the MHC are polymorphic cell surface glycoproteins which bind peptide Ags and display them on the surface of APC for recognition by CD4+ T cells. Though MHC class II molecules are mainly associated with peptides derived from endogenous proteins (1–3), in terms of host defense, class II molecules function primarily to display peptides derived from exogenous Ags. The selectivity of class II molecules for exogenous Ags derives from a distinct intracellular trafficking pathway of class II molecules and distinct intracellular sites in APC for processing of exogenous Ags (4–7).

In the class II pathway, class II α and β chains assemble in the endoplasmic reticulum (ER) with the invariant chain (Ii). A short region of Ii encoded by exon 3 prevents premature binding of peptides in the ER to class II α/β by sterically blocking the Ag binding groove (8, 9). This portion of Ii also appears to act as a surrogate peptide to promote egress of nonamer (α/β–Ii)3 complexes out of the ER (10, 11). Ii targets α/β–Ii to the endosomal compartments where it is selectively removed, in part by acid proteolysis (12–16). The complete dissociation of Ii allows access of the α/β binding groove to exogenous peptides for functional α/β–peptide complex assembly (17). Specialized intracellular vesicles within APC, termed MIIC (5) or CIIV (18), have been implicated as putative sites for final Ii dissociation and peptide loading (6, 18–20). Until recently, details of the molecular nature of peptide loading within this compartment were unknown.

Using a somatic cell mutant approach, we and others have shown that the products of the HLA-DM locus have a critical function in the assembly of MHC class II molecules with cognate peptides (21–24). HLA-DM A and DM B genes map to the class II region of the HLA complex (25) and encode subunits of an unconventional MHC class II heterodimer. HLA-DM mutants are defective in antigen processing; although these mutants express normal levels of class II molecules (22, 26–29), they fail to assemble normal α/β–peptide complexes (30–32). This defect in DM mutants is a direct consequence (33) of an aberrantly high percentage of class II molecules that remain complexed with...
ii-derived class II–associated ii peptides (CLIP) (30–32). CLIP are a nested set of peptides derived from amino acids 81–104 of ii. CLIP-class ii/β complexes are normal intermediates in the MHC class II biosynthetic route (34). DM mutants have a block in a step required for removal of CLIP from class II molecules, and to facilitate loading of cognate peptides (23, 35–37). Presumably this activity of DM is restricted to the peptide loading compartment(s) of APCs. Indeed, HLA-DM has been shown to localize intracellularly to MIIC (38, 39). How DM expression is regulated, how its activity is coordinated with the expression of conventional class II molecules, and what other factors might interact in the class II pathway remain important unresolved problems. However, recent available data suggest that the DM genes are regulated coordinately with class II genes under most conditions (52).

To further analyze the pathway of class II antigen processing, we have isolated additional mutant APCs defective in MHC class II–peptide complex assembly. Here we describe a set of mutants which display a novel class II–deficient phenotype resembling that of DM mutants. However, these mutants are complementary with T2, a cell that is homozygously deleted for HLA-DM and the entire class II region (34, 40, 41). Therefore, the lesions in these new mutants cannot be in the HLA-DM genes, and the affected genes map outside the HLA class II region. We provide evidence that these mutants fail to express sufficient levels of DM heterodimers to effect normal DM function. The results suggest that DM and class II can be differentially regulated, and that this differential regulation has significant effects on MHC class II–cognate peptide assembly.

Materials and Methods

Cell lines and derivation of M utants. M utants 2.2.93 and 2.7.93, and mutants 3.6.95 and 3.4.95, were isolated from ethyl methane sulfonate (EMS) mutagenized 3.1.0/DR 3 and 6.3.6/DR 3, respectively, by immunoselection with anti-DR 3 mAb 7.3.19.1 (42) and rabbit complement. To derive 3.1.0/DR 3 and 6.3.6/DR 3, a 1.2-kb DR B1.0301 cDNA was cloned from B-LCL 8.1.6 into pGEM-4 (Promega Corp., Madison, WI). This was then subcloned into the EcoRI site of pBluescript KS +. (Strategene Inc., La Jolla, CA) to make pDR B1.121. A HindIII/Smal DR B1.0301 cDNA fragment from pDR B1.121 was gel purified and subcloned into the HindIII/ Hpal site of pLX SHD, a retroviral expression vector (43). This was used to obtain clones of the packaging line PA317 (43) to transduce B-LCL 3.1.0 and 6.3.6. DR 3-expressing clones were obtained by 3-d coculture of B-LCL with line PA317 (43) to transduce B-LCL 3.1.0 and 6.3.6. DR 3, an EcoRI/BamHI DR B1.0301 cDNA fragment from pDR B1.121 was gel purified and subcloned into the EcoRI/BamHI site of pLX SHD, a similar retroviral expression vector (44) containing his D. This was used also to derive a clone of the packaging line PA317, which was used to super-infect 6.3.6 for high-level DR 3 expression. Transduced 6.3.6 was doubled selected in 1 mg/ml G418 (GIBCO BRL, Gaithersburg, MD) and 4 mM L-histidinol (Sigma) to obtain a 6.3.6/DR 3 clone. Both progenitors were mutagenized with EMS to induce point mutations before immunoselection (45).

Somatic cell hybrids were prepared by PEG fusion as described (26) and were selected using methotrexate (2 × 10⁻⁷ M) and G418 (1 mg/ml). All hybrids are uncloned, stable lines. For stable transfection of 2.7.93 with DMA, 2.7.93 was transduced with a DMA retroviral packaging line as described for 2.2.93 (22). 2.7.93/DM A and 2.2.93/DM A were obtained by transduction of 2.7.93 and 2.2.93, respectively, with a DMA retroviral packaging line as described for 2.2.93 (22). Stable clones were obtained by selection in 4 mM L-histidinol.

Flow Cytometric Analysis. For immunofluorescent flow cytometry, 0.5 × 10⁶ cells were stained for 1 h at 4°C with saturating amounts of the indicated mAbs in 50 μl R PMI/5% FBS, washed and further stained 1 h at 4°C with 1:100 fluoresceinated goat F(ab')₂ anti-mouse IgG (FITC-GAM) (Sigma). After staining, cells were washed, resuspended in 25 μg/ml propidium iodide (PI) for dye exclusion (viability) gating, and run through FACScan (Becton-Dickinson) and analyzed with REPROMAN. Cells in each panel were analyzed in parallel. Histograms represent immunofluorescence on viable, triple-gated events (forward scatter FSC, side scatter SSC, and PI exclusion).

To obtain DM α-specific anti-serum 8383H, a recombinant fusion protein was constructed by subcloning, from pDM A (22), a 570-bp cDNA fragment encoding the DM α1 and DM α2 in frame into pMAL-c2 (N.E. Biolabs) for bacterial expression. Recombinant fusion protein was purified as described (NEB Instruction manual, Version 3.01). Protein fusion and expression system. 1993) and was used to immunize and boost NZW rabbits for production of anti-serum 8383H.

M HC Class II Dimer Stability Assays. Western immunoblots were stained with a combination of mAb D A 6.147, which recognizes HLA-DR α monomers and HLA-DR α/β dimers (53), and mAb H B 10.A, which recognizes HLA-DR β monomers and HLA-DR α/β dimers (54). For dimer stability assays, nondenatured N-P 40 extracted whole cell lysates were prepared and 1 × 10⁶ cell equivalents per sample were run in SDS-PAGE and transferred to PVDF. Membranes were stained with mAb followed by horseradish peroxidase–anti–mouse IgG and [1²5I] protein-A.

C el Sorting. To fractionate 2.7.93 into mAb 16.23 subpopulations, 10² cells of a 2.7.93 clone were stained with saturating levels of mAb 16.23 in RPMI/5% FBS, washed and stained with FITC-GAM, and then resuspended in
PI for viability gating. Cells were sorted by FACStar® (Becton-Dickinson) by setting collection gates to collect the 10% of the stained population expressing the lowest levels, or highest levels, of the 16.23 epitope to obtain mAb 16.23 stained population expressing the lowest levels, or highest levels, of Dickenson) by setting collection gates to collect the 10% of the tometry.

and were analyzed for mAb 16.23 expression by indirect flow cy-

described (55). Membranes were stained with anti-DM prepared and run in reducing SDS-PAGE and transferred as de-

scribed (55). Membranes were stained with anti-DMα anti-serum 8338H followed by biotinylated goat anti-rabbit IgG (GIBCO BRL) and Streptavidin-HRP (GIBCO BRL) followed by Lumiglo substrate (Kirkegaard and Perry Labs, Gaithersburg, MD).

Western Immunoblots. Boiled, detergent (NP-40) extracted cell lysates from 10⁶ cells/lane or 0.5 × 10⁶ hybrids/lane were prepared and run in reducing SDS-PAGE and transferred as de-
cribed (55). Membranes were stained with anti-DMα anti-serum

3.1.0/DR3- and 6.3.6/DR3 progenitors were muta-
genized with EMS and were immunoselected with mAb 26.4.36 (DR 1/DR 3- and 6.3.6 (DR 1+/DR 3-) were first transduced with DR B1.0301. HLA-DR 3 molecules exhibit reduced binding of certain mAbs, e.g., mAb 16.23 and mAb 7.3.19.1, as a result of changes in occupancy of the peptide binding groove (22, 26). The 3.1.0/DR 3 and 6.3.6/DR 3 progenitors were mutagenized with EMS and were immunoselected with mAb 7.3.19.1. After immunoselection, surviving clones were screened for loss of mAb 16.23 binding and further analyzed for class II alterations suggestive of defects in MHC class II/peptide assembly. Mutants 7.3.19.1 and 3.6.95. Deletions are indicated by horizontal hatched bars on the deleted haplotype and have been previously described for 3.1.0 and 6.3.6 (71). Vertical lines indicate approximate locations of genetic loci and details of the HLA class II region (72). (d) The M-b class II region HLA homozogous deletion in T2 (T × B hybrid line: 721.174 × CEM.T2) (40). The centromeres are to the left. Not to scale.

Figure 1. Derivation of mutan cell lines. (a) Regions of chromosome 6p hemizygously deleted in progenitors 3.1.0 (~40 Mb) and 6.3.6 (~30 Mb) and their positions relative to the HLA complex. (b) The HLA class II region haplotypes of B-LCL 3.1.0, the progenitor of mutant 2.7.93 and mutant 2.2.93 (DMA null) (22). (c) The HLA class II region haplotypes of B-LCL 6.3.6, the progenitor of mutants 3.6.95 and 3.4.95. Deletions are indicated by horizontal hatched bars on the deleted haplotype and have been previously described for 3.1.0 and 6.3.6 (71).

For transient expression experiments, cDNA encoding HLA-DMB and cDNA encoding HLA-DMA were cloned into the expression vector pcDNA1/αMP (Invitrogen Corp., Carlsbad, CA) to obtain plasmids pDMB and pDMA, respectively, as described (22). pcDNA1/αMP contains Polyoma and SV40 origins of replication, allowing for episomal replication. For transient transfections, 2.5 × 10⁶ of the indicated cells were resuspended in 0.5 ml RPMI with 15-25-µg circular pDM B or pDMA or both and were electroporated using 2.2 kV. Samples were cultured for 72 h to regain high viability and then were stained and analyzed by flow cytometry as described in Fig. 2.

Northern Analysis. Total cellular RNA was isolated and northern blots were prepared by standard techniques (56, 57). Probes used for hybridization were labeled with [32P]dCTP using random primers (Boehringer Mannheim Biochemicals, Indianapolis, IN). PCR was used to generate full-length probes from DMA and DMB cDNAs as described (22). The actin probe was a cDNA in a linearized plasmid (58).

Results

Derivation of Mutants 2.7.93, 3.6.95, and 3.4.95. EBV transformed human B-lymphoblastoid cell lines (B-LCL) with hemizygous MHC deletions have been useful as progenitors for immunoselecting recessive mutants which are defective in MHC class II/peptide assembly, including DM mutants previously isolated (21, 22, 26, 27). Using this approach, we derived mutant 2.7.93 from progenitor 3.1.0, which contains an ~40-Mb hemizygous deletion in chromosome 6p, removing one HLA haplotype (59) (Fig. 1). Mutants 3.6.95 and 3.4.95 were similarly derived from progenitor 6.3.6, which contains a smaller, ~30-Mb hemizygous deletion in chromosome 6p, which is overlapped by the 3.1.0 deletion (59) (Fig. 1). As a basis for immunoselection of these mutants, progenitors 3.1.0 (DR1 b) and 6.3.6 (DR1 a+/DR3+) were first transduced with DR B1.0301. HLA-DR 3 molecules exhibit reduced binding of certain mAbs, e.g., mAb 16.23 and mAb 7.3.19.1, as a result of changes in occupancy of the peptide binding groove (22, 26). The 3.1.0/DR 3 and 6.3.6/DR 3 progenitors were mutagenized with EMS and were immunoselected with mAb 7.3.19.1. After immunoselection, surviving clones were screened for loss of mAb 16.23 binding and further analyzed for class II alterations suggestive of defects in MHC class II/peptide assembly. Mutants 2.7.93, 3.4.95, and 3.6.95 were isolated from mutagenized progenitors at frequencies of ~10⁻⁴, consistent with observed frequencies for EMS-induced recessive mutations in other hemizygous loci using equivalent EMS concentrations and standard protocols (55, 60).

Mutants 2.7.93, 3.6.95, and 3.4.95 express C onformationally Altered MHC Class II Molecules at the Cell Surface. The class II cell surface phenotypes of the new mutants were characterized by FACS® analysis using a panel of mAbs
Mutant 2.7.93 expresses conformationally altered class II molecules. Cell surface staining of MHC molecules on mutant 2.7.93. M mutant 2.7.93 (dark line), its progenitor, 3.1.0/DR 3 (thin line), control DMA null mutant, 2.2.93 (heavy dashed), and negative control, T2 (thin dashed), were stained in indirect immunofluorescent flow cytometry using class II conformation-independent mAbs: (a) L-243 (anti-DR); (b) VI-15 (anti-DR); (c) UK8.1 (anti-DR 3, 5, 7); (d) 62.74 (anti-DR 1); and class II conformation-sensitive mAbs: (e) 16.23 (anti-DR 3); (f) 4AA7 (anti-DQ 1); (g) 13.3.84 (anti-DP 4.1); and mAb to class I: (h) W6/32. Only mutant 2.7.93 is labeled to highlight its profile. In b only, T2 is heavy dashed and 2.2.93 is thin dashed.

Figure 2. M mutant 2.7.93 expresses conformationally altered class II molecules. Cell surface staining of MHC molecules on mutant 2.7.93. M mutant 2.7.93 (dark line), its progenitor, 3.1.0/DR 3 (thin line), control DMA null mutant, 2.2.93 (heavy dashed), and negative control, T2 (thin dashed), were stained in indirect immunofluorescent flow cytometry using class II conformation-independent mAbs: (a) L-243 (anti-DR); (b) VI-15 (anti-DR); (c) UK8.1 (anti-DR 3, 5, 7); (d) 62.74 (anti-DR 1); and class II conformation-sensitive mAbs: (e) 16.23 (anti-DR 3); (f) 4AA7 (anti-DQ 1); (g) 13.3.84 (anti-DP 4.1); and mAb to class I: (h) W6/32. Only mutant 2.7.93 is labeled to highlight its profile. In b only, T2 is heavy dashed and 2.2.93 is thin dashed.

The expression of MHC class I appears unaffected (Fig. 2 h). M mutants 3.6.95 and 3.4.95 have similar phenotypes to mutant 2.7.93, although they were derived from a different progenitor, 6.3.6/DR 3 (Fig. 1), and thus are independent of 2.7.93. M mutants 3.6.95 and 3.4.95, like 2.7.93, exhibit substantial loss of the mAb 16.23 epitope (Fig. 6 a), but they also retain levels of MHC HLA-DR and of the DR 3 transgene comparable to those of progenitor 6.3.6/DR 3 (Table 1).

Although these changes resemble those of DM null mutants, there are features of the mAb binding profile that distinguish the new mutants from DMA mutant 2.2.93 and other DM mutants. In particular, whereas DM null mutants are essentially mAb 16.23null, mutant 2.7.93 has the mAb 16.23null peak but also a reproducible mAb 16.23low shoulder (Figs. 2 e and 5). M mutants 3.4.95 and 3.6.95 also exhibit this unusual, bimodal distribution of mAb 16.23 staining (Fig. 6 a), although relative to 2.7.93, mutant 3.4.95 consistently displays a greater proportion of mAb 16.23null cells and 3.6.95 consistently displays a greater proportion of mAb 16.23low cells in their respective populations. Thus, these mutants have related, yet distinguishable phenotypes. The bimodal mAb 16.23 staining pattern in these mutants persists despite repeated subcloning (see below and Fig. 5).

A second significant difference between the new mutants and DM null mutants is evident from cell surface binding patterns with a broader panel of class II mAbs, as shown in Fig. 2 and summarized in Table 1. Whereas DM null mutant 2.2.93 is affected principally in the binding of antibodies recognizing class II polymorphic determinants (the DP monomorphous determinant recognized by mAb B7/21 being the only exception), the new mutants are affected not only for these class II polymorphic determinants but for class II monomorphic determinants as well. For example, HLA-DR levels measured by mAb VI.15 and L-243 staining are diminished (~75% of wild type) on mutant 2.7.93. This pattern differs from that of a DM null mutant 2.2.93 (also 3.1.0/DR 3 derived), in which HLA-DR levels measured by mAb VI.15 and L-243 staining are virtually unchanged (reference 22 and Table 1). In addition, the expression of mutant 2.7.93 of DQ determinants recognized by mAbs SPV.3 and 1A3 is ~25–50% of wild type, and is reduced to levels lower than in DMA null mutant 2.2.93 (Table 1).

In summary, both DM-dependent and DM-independent class II cell surface epitopes are affected in the new mutants. However, there is a greater reduction in the expression of DM-dependent as compared to DM-independent epitopes. These data suggest that the genetic lesions in these mutants have pleiotropic effects: a major effect on DM activity and a lesser effect in cell surface class II expression.

M mutants 2.7.93, 3.6.95, and 3.4.95 express MHC class II Molecules Which Are Unstable in SDS. To assess the extent of cognate peptide binding to class II molecules in these new mutants, we analyzed the stability of their class II dimers in SDS. Cognate peptide binding to class II molecules induces conformational changes in class II dimers which enhance their stability in SDS (61–63). Thus, impaired cognate
### Table 1. MHC Class II mAb Staining of Mutants 2.7.93, 3.4.95, 3.6.95

| mAbs (Antigenic Specificity) | Cells | L-243 (DR) | VI.15 (DR) | 16.23 (DR3) | 7.3.19.1 (DR3) | UK8.1 (DR) | 62.74 (DR1) | 4AA7 (DQ1) | SPVL3 (DQ) | Ia3 (DQ) | 13.3.B4 (DP4.1) | B7/21 (DP) |
|-----------------------------|-------|-----------|------------|------------|---------------|------------|-------------|-------------|-------------|---------|----------------|----------|
| 310/DR3                    | (1.00)| (1.00)    | (1.00)     | (1.00)     | (1.00)        | (1.00)     | (1.00)      | (1.00)      | (1.00)      | (1.00)  | (1.00)        | (1.00)   |
| 2.7.93                     | 0.72 ± 0.03 | 0.74 ± 0.08 | 0.15 ± 0.04 | 0.46 ± 0.04 | 0.64 ± 0.09  | 0.93 ± 0.17 | 0.29 ± 0.03 | 0.26 ± 0.03 | 0.45 ± 0.05 | 0.42 ± 0.03 | 0.47 ± 0.07 | (1.00)   |
| 2.7.93*                    | 1.08 ± 0.04 | 1.08 ± 0.20 | 0.04 ± 0.01 | 0.57 ± 0.04 | 0.81 ± 0.08  | 1.53 ± 0.18 | 0.63 ± 0.14 | 0.63 ± 0.12 | 1.15 ± 0.16 | 0.73 ± 0.11 | 0.70 ± 0.03 | (1.00)   |
| 636/DR3                    | (1.00) | (1.00)    | (1.00)     | (1.00)     | (1.00)        | (1.00)     | (1.00)      | (1.00)      | (1.00)      | (1.00)  | (1.00)        | (1.00)   |
| 3.4.95                      | 0.77 ± 0.07 | 0.92 ± 0.15 | 0.15 ± 0.05 | 0.45 ± 0.20 | 0.77 ± 0.08  | 0.60 ± 0.03 | 0.53 ± 0.06 | 0.42 ± 0.12 | 0.52 ± 0.03 | 0.51 ± 0.25 | 0.49 ± 0.06 | (1.00)   |
| 3.6.95                      | 0.87 ± 0.14 | 0.88 ± 0.15 | 0.30 ± 0.04 | 0.66 ± 0.01 | 0.88 ± 0.02  | 0.66 ± 0.11 | 0.75 ± 0.19 | 0.67 ± 0.05 | 0.83 ± 0.10 | 0.66 ± 0.04 | 0.58 ± 0.08 | (1.00)   |

Cell surface class II levels were determined by staining mutants and their progenitors in indirect immunofluorescent flow cytometry. Data are expressed as a proportional value, relative to wild type (1.00), ± SE of replicate experiments.

*2.2.93 is a DM null mutant (22) included for reference staining.
16.23 sorted subpopulations and analyzing them for mAb 16.23 expression. All sub-clones from both populations reveal a variable, but bimodal distribution of mAb 16.23 staining (Fig. 5b). Within sub-clones, changes in the percent distribution of cells into either population are slow, on the order of 1–5% per week of continuous culture (data not shown). While the proportion of cells in each subpopulation varies, the overall phenotype is stable. Finally, it should be noted that the mean mAb 16.23 staining of the mAb 16.23low subpopulations in these mutants is consistently 50% of the mean of that of the progenitors (Figs. 2e and 6a).

The HLA Class II Homozygous Deletion Mutant T2 Complements the Defect in the New Mutants 2.7.93 and 3.6.95. 3.1.0 and 6.3.6, the progenitors of the new mutants, contain large hemizygous deletions of chromosome 6p which encompass the HLA-DMA and DMB loci (Fig. 1). Thus, the DM genes could be considered candidate sites for the mutations because only a single mutational hit in a gene mapping to the hemizygous region would generate a recessive phenotype. However, as described above, these mutants manifest subtle characteristics distinct from those of DM null mutants. Preliminary observations also suggested that the lesions in these new mutants might not be in the HLA-DM genes. We observed that 2.7.93 and the DMB null mutant 9.5.3 are complementary in somatic cell fusions (2.7.93 × 9.5.3 lane in Fig. 3, and data not shown). In addition, stable transduction of 2.7.93 with DMA alone, using a retroviral vector containing a DMA cDNA, fails to complement the 2.7.93 defect (data not shown), though this same construct complements the DMA null mutant 2.2.93 (22). These data tended to exclude mutations in either DMA or DMB alone.

To determine if the mutations of the new mutants map to either the DMA or DMB locus, we made stable somatic cell hybrids between 2.7.93 and T2, and between 3.6.95 and T2. T2 is homozygously deleted for the entire class II region (41) (Fig. 1) and can contribute neither conventional class II molecules nor DM molecules to somatic cell hybrids. Control hybrids between a 3.1.0/DR3-derived DM null mutant 2.2.93 and T2 are noncomplementary (Fig. 6), as expected. However, both mutants 2.7.93 and 3.6.95 are complemented by T2 in somatic cell hybrids. This is evidenced by restoration to wild-type levels of mAb 16.23 binding (Fig. 6), by a dramatic reduction to wild-type levels of cell surface CLIP (Fig. 6), and by the appearance of SDS-stable class II dimers in cell lysates from these hybrids (data not shown). Therefore, the lesions in these new mutants are recessive, cannot be in the HLA-DM genes, and

![Figure 3](image-url)  
**Figure 3.** Mutant 2.7.93 expresses unstable class II dimers, and the defects in dimer stability in mutant 2.7.93 and DMB null 9.5.3 are complementary. Cell lysates from mutant 2.7.93, and the indicated control cell lines, DMA mutant 2.2.93 and DMB null mutant 9.5.3, and lysates from somatic cell hybrids were run in dimer stability assays essentially as described (35). Western blots were stained with a combination of mAb DA6.147, which recognizes DRα monomer and DRα/β dimers, and H810.A, which reacts with DRβ1 and DRβ3 in monomeric form and in DRα/β dimers.

![Figure 4](image-url)  
**Figure 4.** Mutant 2.7.93 expresses increased levels of class II which are associated with CLIP. (a) Control cells, DMA null mutant 2.2.93 and DMB null 9.5.3, their progenitors 3.1.0/DR3 and 8.1.6, and negative control T2, were stained in indirect immunofluorescent flow cytometry with mAb CER.CLIP.1, which recognizes CLIP associated with HLA-DR molecules. (b) Mutant 2.7.93, control cell DMA mutant 2.2.93, their progenitor, 3.1.0/DR3, and negative control T2, were stained in indirect immunofluorescence with mAb CER.CLIP.

![Figure 5](image-url)  
**Figure 5.** mAb 16.23null and mAb 16.23low sorted subpopulations of 2.7.93-type mutants express differential levels of stable HLA-DR dimers. (a) SDS stability of HLA-DR dimers from cell-sorted mAb16.23null and mAb 16.23low subpopulations of mutant 2.7.93. Western blots were stained with a combination of mAbs DA6.147 and H810.A. (b) Bimodal reexpression of the mAb 16.23 epitope in subclones of the 2.7.93 mAb 16.23null sorted subpopulations. 2.7.93 cells were stained in indirect immunofluorescence with mAb 16.23. Each panel represents mAb 16.23 staining of an individual subclone of 2.7.93 (mAb 16.23null); subclones of the mAb 16.23low subpopulation show similar bimodal reexpression of the mAb 16.23 epitope (data not shown).
map outside the 1 Mb HLA class II region. To verify that these somatic hybrids contain the MHC haplotypes of both input partners, we stained them and controls with the TAP-dependent, HLA-B5-specific antibody mAb 4D12. Stable hybrids 2.7.93 × T2 and 3.6.95 × T2 each express the epitope recognized by mAb 4D12 (data not shown). This reciprocal complementation of the TAP defect in T2 by the 2.7.93 and 3.6.93 partners can only be achieved in trans by contributions from both the T2 (TAP−; B5+) and 2.7.93 or 3.6.95 (TAP+; B5−) MHC haplotypes. Thus, these somatic hybrids contain the chromosome 6p haplotypes of both input partners. Stable hybrids between 3.4.95 and T2 have not yet been obtained.

Mutants 2.7.93, 3.6.95 and 3.4.95 Express Diminished Levels of DM Proteins. The T2 complementation results indicate that the lesion(s) in these new mutants map outside the HLA class II region and thus are neither in the HLA-DM structural genes nor in cis-acting DM regulatory elements. Because the phenotype of these mutants resembles that of DM null mutants, these mutants might have regulatory lesions affecting the expression of DM which could explain their unusual phenotype. Western immunoblots of lysates from the mutants and their progenitors were stained with rabbit polyclonal antisera against DMα (Fig. 7) and DMβ (data not shown) to assess relative levels of DM protein expression. Mutant 2.7.93 expresses no detectable DMα monomer; mutants 3.6.95 and 3.4.95 exhibit significantly reduced (1/5 to 1/10 of wild type) levels of DMα monomer (Fig. 7). Western blots with rabbit polyclonal antisera raised against DMβ cytoplasmic tail similarly reveal that mutants 2.7.93, 3.6.95, and 3.4.95 have reduced DMβ monomer (data not shown). This suggested that the phenotype of these mutants could be explained by deficient DM expression.

DMα monomer is increased in Western immunoblots in lysates from 2.7.93 × T2 and 6.95 × T2 hybrids (Fig. 7), consistent with the ability of T2 to complement the other phenotypic defects in these mutants, as shown above. Because T2 cannot contribute DM molecules in these somatic cell hybrids, the restoration of DM expression must result from provision of a transactive factor by T2.

Both HLA-DMA and -DMB Are Required to Complement the 2.7.93 Defect. As shown above, both DMA and DMB proteins are significantly reduced in 2.7.93, and increased levels of these proteins are observed in lysates made from hybrids of mutants × T2. Therefore, we asked if transfections of DMA and DMB driven by heterologous promotor could alter the 2.7.93-type mutant phenotype. In transient transfection assays, cell surface expression of the mAb 16.23 epitope on mutant 2.7.93 was significantly enhanced by transfection of both DMA and DMB in combination, but not

Figure 6. Cell surface staining of MHC class II and MHC class III/CLIP complexes on 3.4.95 and 3.6.95, and on (2.7.93 × T2) and (3.6.95 × T2). (a and b) Cell surface expression of the mAb 16.23 epitope and of MHC class II-CLIP complexes on 6.3.6/DR3-derived mutants 3.4.95 and 3.6.95. (a) Negative control DMA null mutant 2.2.93, mutants 3.6.95 and 3.4.95, and progenitor 6.3.6/DR3, were stained by indirect immunofluorescence with mAb 16.23. Subclones of 3.4.95 and 3.6.95 manifest consistent bimodal expression of the mAb 16.23 epitope as seen in mutant 2.7.93 (data not shown). (b) Negative control 6.1.6 (71), progenitor 6.3.6/DR3, positive control, DMA null mutant 2.2.93, and mutants 3.6.95 and 3.4.95, were stained by indirect immunofluorescence with anti-class II-CLIP mAb I-5, which with HLA class II molecules, (c–f) Cell surface expression of the mAb 16.23 epitope and of MHC class II-CLIP complexes on somatic cell hybrids (c) Negative control hybrid (2.2.93 × T2), positive control hybrid (3.1.0/DR3 × T2), and hybrids (2.7.93 × T2) and (3.6.95 × T2); and (d) control hybrid (3.1.0/DR3 × T2), and hybrids (2.7.93 × T2) and (3.6.95 × T2); and (e) negative control (T2), negative control somatic cell hybrids (2.93 × T2), positive control hybrids (3.1.0/DR3 × T2); and hybrids (2.7.93 × T2) were stained in indirect immunofluorescent flow cytometry using the indicated mAbs.

Figure 7. DM protein levels are reduced in 2.7.93-like mutants. Whole cell lysates from the indicated cells and somatic cell hybrids were run in denaturing SDS-PAGE and analyzed in Western immunoblots by staining with 8338H, a rabbit polyclonal anti-DMA antisera which recognizes HLA-DMA monomer, as described in Materials and Methods.
Table 2. Transient DM Expression Assay: Restoration of the mAb 16.23 Epitope

| Cells          | Genotype   | Transfected DNA (Transient) | N. trials | Δ%16.23+ cells
|----------------|------------|-----------------------------|-----------|----------------
| 2.2.93¹       | DMA−/−     | pDMA                        | n = 15    | 4–19
| 2.2.93        | DMA−/−     | pDMB                        | n = 15    | 0
| 9.5.3¹        | DMB−/−     | pDMA                        | n = 12    | 0
| 9.5.3         | DMB−/−     | pDMB                        | n = 12    | 3–15
| 2.7.93        | ??         | pDMA                        | n = 3     | 0
| 2.7.93        | ??         | pDMB                        | n = 2     | 0
| 2.7.93        | ??         | pDMA + pDMB                 | n = 1     | 11
| 2.7.93/DMA²   | DMA wt     | pDMB                        | n = 2     | 11–13

*Cells were stained and analyzed by indirect immunofluorescent flow cytometry: Δ%16.23+ cells = % cells in R2 (mAb 16.23+) of test population - % cells in R2 of negative control (untransfected) population.

¹DM null mutants 2.2.93 and 9.5.93 (22) included as controls.

²2.7.93 stably transduced with DMA.

by transfection of DMA or DMB individually (Table 2). This is consistent with the finding that stable transfection of 2.7.93 with DMA cDNA alone failed to complement the 2.7.93 defect. Furthermore, this result is consistent with the complementation observed between 2.7.93 and the DMB mutant 9.5.3 (data not shown).

Mutants 2.7.93, 3.6.95, and 3.4.95 Express Diminished Levels of DM mRNA. The transfection results, described above, suggested that DM mRNA levels might be deficient in these mutants. Therefore, DM mRNA levels in the mutants were analyzed by Northern blotting (Fig. 8). These studies confirmed that DM levels were altered in the mutants, and also indicated a phenotypic difference between 2.7.93 on the one hand, and mutants 3.4.95 and 3.6.95 on the other. In 2.7.93, both DMA and DMB levels were markedly reduced, to 10–20% of the levels of progenitor 3.1.0/DR3 (Fig. 8, and data not shown). In mutants 3.4.95 and 3.6.95, DMB levels were likewise reduced to the ~10% level, but DMA levels were only modestly reduced. Thus, the primary defect in 2.7.93 appears to be a reduction in both DMA and DMB mRNA, whereas in 3.4.95 and 3.6.95 the principal defect appears to be a reduction in DMB mRNA. These reductions in DM mRNA provide a reasonable basis for the reductions in DM protein and the secondary manifestations of DM deficiency in the mutants, described above. It is likely that the reduction in DMα protein in 3.4.95 and 3.6.95 results in part from accelerated turnover of DMα protein because of the relative deficiency of the DMβ subunit, as is common when one subunit of a dimeric protein is absent (74).

Discussion

The molecular requirements for the association of antigenic peptides with MHC class II molecules have been more clearly elucidated in the past few years. In particular, the accessory molecule HLA-DM has been shown to provide a critical role in the maturation of functional class II–peptide complexes (21, 22, 24, 54). However, the factors involved in regulating DM expression and its catalytic activity remain to be determined. The importance of DM in proper class II presentation, the presence of typical class II promotor elements in the DM regulatory regions, and the fact that γ-interferon induces DM expression, all imply coordinate expression of DM with other class II molecules. We describe here a set of novel APC mutants which exhibit discoordinate expression of conventional MHC class II molecules and the accessory molecule HLA-DM.

The mutants described in this paper have a phenotype that bears a marked resemblance to that of DM null mutants. In particular, their cell surface class II molecules are predominantly associated with CLIP. Our data also suggest that the phenotype of these mutants directly results from a deficiency in DM expression. However, the defects in two of the mutants tested so far do not map to the HLA-DM loci; in somatic cell hybrids, these mutants are complementary with T2, a fusion partner that contains a homozygous deletion encompassing HLA-DM and -DMB and the entire HLA class II region. Thus the lesions for 2.7.93 and 3.6.95 cannot be in the HLA-DMA or -DMB structural genes nor in any genes in the class II region, including the conventional class II genes. Complementation of the mutant defect in trans with T2 also rules out mutations in DMα-regulatory regions.

The locations of the genes affected in these mutants are not known with certainty. However, their observed frequency in EMS mutagenized progenitors 3.1.0 and 6.3.6 is ~10−4; this is the expected frequency for monozigous loss, and is several orders of magnitude higher than expected for dizygous loss (60). Thus, the genetic lesions in these mutants most likely map to the regions of hemizygous deletions in 3.1.0 and 6.3.6. The hemizygous deletions in 3.1.0
and 6.3.6 encompass ~40 Mb and ~30 Mb, respectively, of chromosome 6p (Fig. 1); therefore the affected gene(s) likely map to these regions of chromosome 6p, but outside the class II region.

Although the defects in mutants 2.7.93 and 3.6.95 do not map to the DM loci, the dramatic reductions in DM proteins and mRNAs suggest that their phenotype results from deficient DM expression. This is evidenced by the fact that class II molecules are expressed but are unstable in SDS, that class II molecules remain complexed with CLIP peptide, and that there is a marked reduction of DM-dependent mAb epitopes on class II molecules which exceeds the reduction in class II levels. Thus, class IIαβ-II complexes are being expressed in the absence of adequate levels of DM for removal of CLIP. Consistent with this, transient transfections of 2.7.93 with DMA and DMB together, but not singly, lead to an increase in the level of mAb 16.23 binding (Table 2). Although all measures of DM activity indicate that each mutant has a major deficiency in DM expression, the lesions also modestly affect class II expression, as indicated by decreased levels of cell surface class II molecules (Fig. 2 and Table 1) and class II mRNAs (Muczynski, K., and B. Arp, unpublished data). These reductions in class II expression are generally not seen in DM structural gene mutants, such as mutant 2.2.93 (Table 1). It appears from these results that the lesions in the new mutants differentially affect DM versus conventional class II expression.

The complementation data suggest that the disproportionate loss of DM expression relative to conventional class II expression in these mutants results from recessive mutations in a transactive factor(s). Previous reports have indicated that DM and class II genes are coordinately regulated. Both class II and HLA-DM transcription are induced by IFN-γ (25, 64, 65), and it has been proposed that the S-X-Y regulatory regions of DM genes function similarly to class II regulatory elements (52). Furthermore, HLA-DM and conventional class II transcription are coordinately regulated in all known cases of class II immunodeficiency (66), in vitro class II transcription mutants (67, 68), and in mutants defective in IFN-γ-induced class II transcription (69). The mutants described here thus provide evidence suggesting that DM and class II expression can be differentially regulated by a trans-acting factor. The existence of an MHC class II isotype-specific trans-acting factor has also been recently described for HLA-DQ (73).

While mutants 2.7.93, 3.6.96, and 3.4.95 all express essentially undetectable levels of DMB mRNA, they express different levels of DMA mRNA, suggesting that different transactive factors may be affected in this set of mutants. Consistent with this, preliminary complementation studies suggest that at least two different factors are affected in the three mutants (Rak, J., unpublished data). How these factors differentially regulate DM and class II mRNA abundance is currently under investigation.

Perhaps the most surprising and distinguishing aspect of the mutants described here is the bimodal mAb 16.23 staining pattern observed in clonal populations. Our data show that expression of the mAb 16.23 epitope on DR3 molecules on these mutants is distributed into distinct 16.23null and 16.23low sub-populations. This bimodal mAb 16.23 staining profile is specific for the DR3 expressed by these mutants; it is not observed with other DM-sensitive mAbs to DQ and DP, nor with mAbs recognizing class II-CLIP. Altered levels of expression of the mAb 16.23 determinant by sub-populations of these mutants do, however, correlate with differences in other measures related to DM activity, i.e., SDS-stability of both DR1 and DR3 dimers (Figs 3, 5) and of DQ and DP dimers (data not shown). Thus, the bimodality observed for 16.23 expression by these mutants is most likely related to their reduced levels of DM expression. This is consistent with preliminary data which indicate that the bimodality for the 16.23 epitope in 2.7.93 is correlated with bimodality for DM expression (Anderson, S., S. Fling, and D. Pious, manuscript in preparation). The biphasic phenotype is suggestive of a threshold effect at the level of DM expression.

The bimodal expression of a DM-dependent, DR3 class II determinant is not unprecedented; it is similar to that described for T2/DR3 transfected with DM (51). Clonal populations of T2/DR3 transfected with DM also stain in a bimodal distribution with a DM-sensitive mAb, in this case mAb CER.CLIP.1 (51). This bimodal profile is specific for DR3 expressed in T2; it is not observed in T2/DR4 nor T2/DR11. As with the staining profile of the mutants described here, the bimodal staining profile of T2/DR3/DQ with the DM-sensitive mAb CER.CLIP.1 cannot be removed by repeated subcloning (51). Anti-CLIP staining on the mutants described here is not distributed into clearly

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**Figure 8.** DNA and DMB mRNA levels are reduced in 2.7.93-like mutants. Total cellular RNA from the indicated cells were analyzed in Northern blots. Full-length probes from DMA and -B cDNAs were labeled with [32P]dCTP and used for hybridization. Reprobing of Northern blots. Full-length probes from DMA and -B cDNAs were labeled with [32P]dCTP and used for hybridization. Reprobing of Northern blots with actin (not shown) indicated equal loading of RNA to all lanes.
distinct CLIP+/- subpopulations, unlike anti-CLIP staining on T2/DR3/DM. This difference most likely results from the ability of the anti-CLIP mAbs used in this study to recognize CLIP in association with each of the four class II alleles (DR1, DR3, DQ1, DP4.1) expressed by these mutants, in contrast, T2/DR3/DM expresses only DR3. The bimodal distribution of DM-dependent epitopes seen specifically on DR3 molecules in these mutants and in T2/DR3/DM could be related and may suggest that the requirements for DM may differ for DR3 and other class II molecules. Differences between DR3 and other class II molecules in their respective affinities for CLIP have been shown (70).

The mutants described in this report appear to define a gene or genes required to maintain coordinated expression of HLA-DM and conventional class II molecules in APC. For the majority of cells in a clonal population of these mutants, the expression of DM appears to be below a threshold required to remove CLIP from class II molecules, and to catalyze their loading with cognate peptides. Overall, our data show that even modest perturbations in the coordinated expression of DM and class II can result in profound differences in the level of functional class II-peptide complexes ultimately expressed by APC. The significant influence of DM on the spectrum of peptides associated with MHC class II suggests the interesting possibility that such dysregulation of DM expression may contribute to some level of immune dysfunction. Identification of the factors deficient in these mutants should help to identify components which regulate expression of DM. That the genes defective in these mutants have effects on both DM and class II expression suggests the defective genes may also regulate other components of the MHC class II antigen processing pathway.

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References

1. Lampson, L.A., and R. Levy. 1980. Two populations of HLA-like molecules on a human B cell line. J. Immunol. 125:293–299.
2. Chicz, R.M., R.G. Urban, J.C. Gorga, D.A.A. Vignali, W.S. Lane, and J.C. Strominger. 1993. Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles. J. Exp. Med. 175:27–47.
3. Hunt, D.F., H. Michel, T.A. Dickinson, J. Shabanowitz, A.L. Cox, K. Sakaguchi, I. Appella, H.M. Grey, and A. Sette. 1992. Peptides presented to the immune system by the murine class II major histocompatibility complex molecule I-A4. Science (Wash. DC). 256:1817–1820.
4. Brodsky, F.M., and L.E. Guagliardi. 1991. The cell biology of antigen processing and presentation. Annu. Rev. Immunol. 9:707–744.
5. Peters, P.J., J.J. Neefjes, V. Oorschot, H.L. Ploegh, and H.J. Geuze. 1991. Segregation of MHC class II molecules from MHC class I molecules in the Golgi complex for transport to lysosomal compartments. Nature (Lond.). 349:669–676.
6. Neefjes, J.J., V. Stollorz, P.J. Peters, H.J. Geuze, and H.L. Ploegh. 1990. The biosynthetic pathway of MHC class II but not class I molecules intersect the endocrine route. Cell. 61:171–183.
7. Guagliardi, L.E., B. Koppelman, J.S. Blum, M.S. Marks, P. Cresswell, and F.M. Brodsky. 1990. Co-localization of molecules involved in antigen processing and presentation in an early endocytic compartment. Nature (Lond.). 343:133–139.
8. Teyton, L., D. O'Sullivan, P.W. Dickson, V. Lottau, A. Sette, P. Fink, and P.A. Peterson. 1990. Invariant chain distinguishes between the exogenous and endogenous antigen presentation pathways. Nature (Lond.). 348:39–44.
9. Roche, P.A., and P. Cresswell. 1990. Invariant chain association with HLA-DR molecule inhibits immunogenic peptide binding. Nature (Lond.). 345:615–618.
10. Romagnoli, P., and R. Germain. 1994. The CLIP region of invariant chain plays a critical role in regulating major histocompatibility complex class II folding, transport, and peptide occupancy. J. Exp. Med. 180:1107–1113.
11. Roche, P.A., M.S. Marks, and P. Cresswell. 1991. Formation of a nine-subunit complex by HLA class II glycoproteins and the invariant chain. Nature (Lond.). 354:392–394.
12. Lottau, V., L. Teyton, A. Peleraux, T. Nilsen, L. Karlson, S.L. Schmid, V. Quaranta, and P.A. Peterson. 1990. Intracellular transport of class II molecules directed by invariant chain. Nature (Lond.). 348:600–604.
13. Bakke, O., and B. Dobberstein. 1990. MHC class II-associated invariant chain contains a sorting signal for endosomal compartments. Cell. 63:707–716.
14. Blum, J.S., and P. Cresswell. 1988. Role for intracellular proteases in the processing and transport of class II HLA antigens. Proc. Natl. Acad. Sci. USA. 85:3975–3979.
15. Nguyen, Q.V., W. Knapp, and R.E. Humphreys. 1989. Inhibition by leupeptin and antipain of the intracellular proteol-
...lys of II. Hum. Immunol. 24:153–163.
16. Maric, M.A., M.D. Taylor, and J.S. Blum. 1994. Endosomal aspartic proteases are required for invariant-chain processing. Proc. Natl. Acad. Sci. USA. 91:2171–2175.
17. Cresswell, P. 1994. Assembly, transport and function of MHC class II molecules. Annu. Rev. Immunol. 12:259–293.
18. Amigorena, S., J.R. Drake, P. Webster. 1994. Transient dissociation of HLA-DM mutants from the major histocompatibility complex. Science (Wash. DC). 266:1569–1573.
19. Qiu, Y., X. Xu, A. Wandinger-Ness, D.P. Dalke, and S.K. Miller. 1991. L-histidinol provides effective selection of retrovirus-vector-transduced keratinocytes without impairing their proliferation potential. J. Exp. Med. 174:1607–1615.
20. Tulp, A., D. Verwoerd, B. Dobberstein, H.L. Ploegh, and J. Pieters. 1994. Isolation and characterization of the intracellular MHC class II compartment. Nature (Lond.). 369:113–120.
21. Oiu, Y., X. Xu, A. Wandinger-Ness, D.P. Dalke, and S.K. Miller. 1991. L-histidinol provides effective selection of retrovirus-vector-transduced keratinocytes without impairing their proliferation potential. J. Exp. Med. 174:1607–1615.
22. Fling, S.P., B. Arp, and D. Pious. 1994. HLA-DMA and DM B genes are both required for MHC class II/peptide complex formation in antigen-presenting cells. Nature (Lond.). 368:554–558.
23. Denzin, L.K., and P. Cresswell. 1995. HLA-DM induces CLIP dissociation from MHC class II αβ dimers and facilitates peptide loading. Cell. 82:155–165.
24. Ceman, S., R.A. Rudersdorf, J.M. Petersen, and R. DeMars. 1995. HLA-DMA and DM B are the only genes in the class II region of the human MHC needed for class II-associated antigen processing. J. Immunol. 159:2545–2556.
25. Kelly, A.P., J.J. Monaco, S. Cho, and J. Trowsdale. 1991. A new human HLA class II-related locus, DM. Nature (Lond.). 353:571–576.
26. 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.). 343:71–74.
27. Mellins, E., S. Kempin, L. Smith, T. Monji, and D. Pious. 1991. A gene required for class II-restricted antigen presentation maps to the major histocompatibility complex. J. Exp. Med. 174:1067–1061.
28. Riberdy, J.M., and P. Cresswell. 1992. The antigen-processing mutant T2 suggests a role for MHC-linked genes in class II antigen presentation. J. Immunol. 148:2856–2859.
29. Ceman, S., R.A. Rudersdorf, E.O. Long, and R. DeMars. 1992. HLA class II deletion mutant expresses normal levels of transgene encoded class II molecules that have abnormal conformation and impaired antigen presentation ability. J. Immunol. 149:754–761.
30. Sette, A., S. Ceman, R.T. Kubo, K. Sakaguchi, E. Appella, D.F. Hunt, T.A. Davis, H. Michel, J. Shabanowitiz, R. Rudersdorf, et al. 1992. Invariant chain peptides in most HLA-DR molecules of an antigen-processing mutant. Science (Wash. DC). 258:1801–1804.
31. Riberdy, J.M., J.E. Newcomb, M.J. Surman, J.A. Barbosa, and P. Cresswell. 1992. HLA-DR molecules from an antigen-processing mutant cell line are associated with invariant chain peptides. Nature (Lond.). 360:474–477.
32. Mellins, E., P. Cameron, M. Amaya, S. Goodman, D. Pious, L. Smith, and B. Arp. 1994. A mutant human histocompatibility leukocyte antigen DR molecule associated with invariant chain peptides. J. Exp. Med. 179:541–549.
33. Monji, T., A.L. McCormack, J.R. Yates, D. Pious. 1994. Invariant-cognate peptide exchange restores class II dimer stability in HLA-DM mutants. J. Immunol. 153:4468–4477.
34. Riberdy, J.M., R.R. Avva, H.J. Geuze, and P. Cresswell. 1994. Transport and intracellular distribution of MHC class II molecules and associated invariant chain in normal and antigen-processing mutant cell lines. J. Cell Biol. 125:1225–1237.
35. Sloan, V.S., P. Cameron, G. Porter, M. Gammon, M. Amaya, E. Mellins, and D.M. Zaller. 1995. Mediation by HLA-DM of dissociation of peptides from HLA-DR. Nature (Lond.). 375:802–806.
36. Sherman, M.A., D.A. Weber, and P.E. Jensen. 1995. DM enhances peptide binding to class II MHC by release of invariant chain-derived peptide. Immunity. 3:197–205.
37. Weaber, D.A., B.D. Evavold, and P.E. Jensen. 1996. Enhanced dissociation of HLA-DR-bound peptides in the presence of HLA-DM. Science (Wash. DC). 274:618–620.
38. Sanderson, F., M.J. Kleijmeer, A. Kelly, D. Verwoerd, A. Tulp, J.J. Nefles, H.J. Geuze, and J. Trowsdale. 1994. Accumulation of HLA-DM, a regulator of antigen processing, in MHC class II compartments. Science (Wash. DC). 266:1566–1569.
39. Karlsson, L., A. Péléraux, R. Lindstedt, M. Lijiedahl, and P.A. Peterson. 1994. Reconstitution of an operational MHC class II compartment in nonantigen-presenting cells. Science (Wash. DC). 266:1569–1573.
40. Sailer, R.D., D.N. Howell, and P. Cresswell. 1985. Genes regulating HLA class I antigen expression in T-B lymphoblast hybrids. Immunogenetics. 21:235–246.
41. Sailer, R.D., and P. Cresswell. 1986. Impaired assembly and transport of HLA-A and -B antigens in a mutant T × B cell hybrid. EMBO J. 5:943–949.
42. Koning, F., I. Schrueder, M. Giphard, and H. Bruning. 1984. A mouse monoclonal antibody detecting a DR-related MT2-like specificity: serology and biochemistry. Hum. Immunol. 9:221–230.
43. Miller, A.D., and G.J. Rosman. 1989. Improved retroviral vectors for gene transfer and expression. Biotechniques 7:980–990.
44. Stockschlaeder, M.A., R. Storb, W.R. Osborne, and A.D. Miller. 1991. L-histidinol provides effective selection of retrovirus-vector-transduced keratinocytes without impairing their proliferative potential. Hum. Genet. Th. 2:33–39.
45. Pious, D., C. Soderland, and P. Gladstone. 1977. Induction of HLA mutations by chemical mutagens in human lymphoid cells. Immunogenetics. 4:437–448.
46. 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. 79:1235–1239.
47. Bodmer, J.G., J.M. Hedges and J. Lindsay. 1985. Studies of monoclonal antibodies to the HLA-D region: DQ w1, DR w52. In Histocompatibility Testing. E.D. Albert, M.P. Baur, and W.R. Mayr, editors. Springer Verlag, Berlin. pp. 432–438.
48. Johnson, J., T. Meo, G. Riethmuller, D. Schendel, and R. Wank. 1982. Direct demonstration of an HLA-DR allootypic determinant on the low molecular weight (beta) subunit using a mouse monoclonal antibody specific for DR3. J. Exp. Med. 156:104–111.
49. Clark, E.A., G.L. Shu, B. Lücher, K.E. Draves, J. Banchereau, J.A. Ledbetter, and M.A. Valentine. 1989. Activation of human B cells. Comparison of the signal transduced by IL-4 to four different competence signals. J. Immunol. 143:3873–3880.
50. Viken, H.D., G. Gaudernack, and E. Thorsby. 1989. Characterization of a monoclonal antibody recognizing a polymorphic epitope mainly on HLA-DPw2 and DPw4 molecules. Tissue Antigens. 34:250–259.

51. Denzin, L.K., N.F. Robbins, C. Carboy-Newcombe, and P. Cresswell. 1994. Assembly and intracellular transport of HLA-DM and correction of the class II antigen-processing defect in T2 cells. Immunity. 1:595–606.

52. Westerheide, S.D., P. Louis-Pence, D. Ping, X.F. He, and T. Yokoshi. 1992. HLA-DM and HLA-DMB gene expression functions through the conserved S-X-Y region. J. Immunol. 158:4812–4821.

53. Pious, D., S. Fling, and T. Monji. 1994. Genetic approaches to class II restricted antigen processing and presentation. Annu. Rev. Immunol. 11:403–450.

54. Denzin, L.K., N.F. Robbins, C. Carboy-Newcombe, and P. Cresswell. 1994. Assembly and intracellular transport of HLA-DM and correction of the class II antigen-processing defect in T2 cells. Immunity. 1:595–606.

55. Guy, K., V. Van Heyningen, B.B. Cohen, D.L. Deane, and C.M. Steel. 1982. Differential expression and serologically distinct subpopulations of human Ia antigens detected with monoclonal antibodies to Ia alpha and beta chains. Eur. J. Immunol. 12:942–946.

56. Glisin, V., R. Crkuenjakov, and C. Byus. 1974. Ribonucleic acid isolated by cesium centrifugation. Biochemistry. 13:2633–2637.

57. Chirgwin, J.M., A.E. Przybyla, R.J. MacDonald, and W.J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry. 18:5294–5299.

58. Kovats, S., S. Drover, W.H. Marshall, D. Freed, P.E. Whiteley, G.T. Nepom, and J.S. Blum. 1994. Coordinate defects in HLA class II expression and antigen presentation in bare lymphocyte syndrome. J. Exp. Med. 179:2017–2022.

59. Glimcher, L.H., and C.J. Kara. 1992. Sequences and factors a guide to MHC class II transcription. Annu. Rev. Immunol. 10:13–49.

60. Pious, D., J. 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. 162:1193–1207.

61. Galán, V., R. Crkvenjakov, and C. Byus. 1974. Ribonucleic acid isolated by cesium centrifugation. Biochemistry. 13:2633–2637.

62. Chirgwin, J.M., A.E. Przybyla, R.J. MacDonald, and W.J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry. 18:5294–5299.

63. Germain, R.N., and L.R. Hendriks. 1991. MHC class II structure, occupancy and surface expression determined by post-endothelial reticulum antigen binding. Nature (Lond.). 353:134–139.

64. Peterlin, B.M., T.A. Gonwa, and J.D. Stobo. 1984. Expression of HLA-DR by a human monocyte cell line is under transcriptional control. J. Mol. Cell. Immunol. 1:191–200.

65. Collins, T., A.J. Korman, C.T. Wake, J.M. Boss, D.J. Kappes, W. Fiers, K.A. Ault, M.A. Gimbrone, J.L. Strominger, and J.S. Pober. 1984. Immune interferon activates multiple class II major histocompatibility complex genes and the associated invariant chain gene in human endothelial cells and dermal fibroblasts. Proc. Natl. Acad. Sci. USA. 81:4917–4921.

66. Stowe, A., M. Holbrook, J. Miler, and E. Appella. 1995. Binding of major histocompatibility complex class II to the invariant chain-derived peptide, CLIP, is regulated by allelic polymorphism in class II. J. Exp. Med. 181:677–683.

67. Levine, F., H. Ehrlich, B. Mach, R. Leach, R. White, and D. Pious. 1985. Deletion mapping of HLA and chromosome 6p genes. Proc. Natl. Acad. Sci. USA. 82:3741–3745.

68. Campell, R.D., and J. Trowsdale. 1993. Map of the MHC. Immunol. Today. 7:349–352.

69. Douhan, J., R. Lieberson, J.H.M. Knoll, H. Zhou, and L.H. Glimcher. 1997. HLA-DMA and HLA-DMB gene expression functions through the conserved S-X-Y region. J. Immunol. 158:4812–4821.

70. Cotner, T., H. Charbonneau, E. Melling, and D. Pious. 1989. mRNA abundance, rather than differences in subunit assembly, determine differential expression of HLA-DR beta 1 and -DR beta 3 molecules. J. Biol. Chem. 264:11107–11111.