Evolutionary Conservation of Major Histocompatibility Complex–DR/Peptide/T Cell Interactions in Primates

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Summary

Many major histocompatibility complex (MHC) polymorphisms originate from ancient structures that predate speciation. As a consequence, members of the \( \text{Mhc-DRB1}^*03 \) allelic lineage are not only present in humans but in chimpanzees and rhesus macaques as well. This emphasizes that \( \text{Mhc-DRB1}^*03 \) members must have been present in a common ancestor of these primate species that lived about 30 million years ago. Due to the accumulation of genetic variation, however, alleles of the \( \text{Mhc-DRB1}^*03 \) lineage exhibit species-unique sequences. To investigate the biological importance of such conservation and variation, we have studied both the binding and antigen presentation capacity of various trans-species \( \text{Mhc-DRB1}^*03 \) lineage members. Here we show that p3-13 of the 65-kD heat-shock protein (hsp65) of \( \text{Mycobacterium leprae} \) and \( \text{M. tuberculosis} \) binds not only to HLA-DR17(3) but also to some chimpanzee and rhesus macaque class II-positive cells. Comparison of the corresponding human, chimpanzee, and rhesus macaque \( \text{Mhc-DRB1}^*03 \) lineage members revealed the presence of uniquely shared amino acid residues, at positions 9–13 and 26–31, of the antigen-binding site that are critical for p3-13 binding. In addition it is shown that several nonhuman primate antigen-presenting cells that bind p3-13 can activate HLA-DR17-restricted T cells. Certain amino acid replacements, however, in \( \text{Mhc-DRB1}^*03 \) lineage members did not influence peptide binding or T cell recognition. Therefore, these studies demonstrate that some polymorphic amino acid residues (motifs) within the antigen-binding site of MHC class II molecules that are crucial for peptide binding and recognition by the T cell receptor have been conserved for over 30 million years.

Peptides are able to bind to specificity pockets within the peptide-binding site of MHC molecules (1–3). The proposed model for the structure of MHC class II molecules (4), which has been determined based on the resolution of the structure of HLA class I (5), shows that most polymorphic amino acid residues map within the peptide-binding site. Mhc polymorphism thus will result in the differential selection of peptides available for T cell recognition.

Mycobacterial heat-shock proteins (hsp) are dominant T cell antigens in the response of healthy as well as mycobacterial disease–affected individuals (6–8). The antigenicity of, but also the high homology between, several mammalian and bacterial hsp has led to the hypothesis that T cells initially triggered by an infection may become crossreactive with self-peptides. Eventually this results in, or may even prolong, autoimmune responses at local sites of inflammation (9). The best characterized mycobacterial hsp is hsp65 (10), which is recognized in the context of several different HLA-DR molecules by CD4-positive, antigen-specific T cells (7, 8).

Previously it was reported that hsp65 p3-13 is immunodominant in the mycobacterium-specific T cell response of HLA-DR17(3)–positive individuals and is not recognized in the context of any other HLA-DR molecule (11). This DR17-restricted T cell recognition most probably arises from the fact that p3-13 binds to motifs uniquely present in HLA-DR17 molecules (12).

Comparison of nucleotide sequences showed a high degree of similarity between some nonhuman primate Mhc alleles and certain HLA orthologs (13–17). The degree of similarity between related Mhc class II alleles of different spe-
cies is always higher than that found for alleles grouping in distinct lineages of the same species, as is consistent with the trans-species hypothesis (18). This hypothesis states that closely related Mhc alleles in different species originate from common ancestral alleles, predating the divergence of these species. Trans-species alleles that group into a lineage are generally unique to a species due to the accumulation of genetic variation, mainly caused by point mutations or crossing over events (19–21).

In this study we have investigated the functional importance of these conserved motifs that are present in the diverged members of the Mhc-DRB1*03 allelic lineage of humans, chimpanzees (Pan troglodytes), and rhesus macaques (Macaca mulatta). The capacity of different primates Mhc-DRB1*03 members to bind and present hsp65 p3-13 was analyzed using non-human primate–derived EBV-B lymphoblastoid cell lines (BLCL) as “biomutant” APC. As the corresponding nucleotide sequences of the Mhc-DRB molecules of these cells have been determined (20, 21), it is now possible to pinpoint the essential Mhc-DRB1*03-encoded residues involved in the binding of hsp65 p3-13. Moreover, the functionality of the p3-13 binding was analyzed in T cell stimulation assays using HLA-DR17-restricted, hsp65 p3-13-reactive human T cell clones and nonhuman primate–derived EBV-BLCL as APC.

Materials and Methods

Synthetic Peptides. Synthesis of p3-13 (KTIAYDEEARR) and p307-319 (PKYVKQNTKLKLAT) has been described (12). In short, peptides were made by standard solid-phase methods on a peptide synthesizer using Fmoc amino acid pentfluorophenyl esters (Cambridge Research Biochemicals Ltd., Cambridge, England). The long chain biotinylated analogues of the peptides were made by coupling of 6-(Fmoc amino) hexanoyl acid and biotin (Serva, Heidelberg, Germany), respectively, at the end of the synthesis. The peptides were purified by gel filtration chromatography on a Sephadex G-25 (superfine) column (Pharmacia, Uppsala, Sweden) followed by reversed-phase HPLC (C18) purification, and analyzed by fast atom bombardment mass spectrometry.

Binding Assay. In the binding assay (12, 22), EBV-BLCL (3 × 10^6/sample) were incubated with the biotinylated peptide (50 μM) at 37°C for 4 or 20 h. As a control, cells were labeled in each experiment with a biotinylated mAb specific for HLA-DR (Becton Dickinson & Co., Mountain View, CA) at 4°C for 1 h. Peptide or anti-DR preincubation were followed by labeling with FITC-avidin D (10 μg/ml; 100 μl; Vector Labs, Burlingame, CA) at 4°C for 30 min. Incubation with FITC-avidin D was followed by incubation of biotinylated anti-avidin D (10 μg/ml; 100 μl; Vector Labs) and FITC-avidin D again. After each incubation, excess reagents were washed off at 4°C using PBS containing 0.1% BSA. Stained cells were analyzed by flow cytometry on a FACScan® analyzer (Becton Dickinson & Co.). Dead cells were excluded from the analysis by propidium iodide staining. To measure the relative amount of FITC-avidin D bound, the mean fluorescence of 5,000 stained cells was determined. Background fluorescence, measured in the absence of peptide, was subtracted.

mAbs. The following mAbs were used in this study: anti-DR (B8.11.2); anti-class I (W6/32); anti-class II (1C2); anti-DQ (SPVL3); anti-DP (B7/21); anti-DR52 (7.3.19.1). All antibodies were used in a final concentration comparable with the ascites solution diluted 1:100 (in the binding assays) and 1:200 (in T cell proliferation assays). As a control, nonimmune mouse ascites were tested.

T Cell Proliferation Assays. Proliferation was assayed by mixing 10^4 T cells, irradiated DR-matched allogeneic PBMC (5 × 10^5/well), or EBV-BLCL (3 × 10^5/well) and antigen in 96-well flat-bottomed microtiter plates in triplicate. After 66 h of culture, 1 μCi [3H]thymidine was added to each well, and 18 h later cells were collected on glass fiber strips, and the radioactivity incorporated into the DNA was determined by liquid scintillation counting. Mean and SEMs were computed from the cpm of triplicate tests.

Competition Experiments. Inhibition of activation of the HLA-DR17-restricted, p3-13-reactive T cell clone CAAp15-1-1 was studied in T cell proliferation assays by mixing 10^4 T cells, irradiated PBMC (5 × 10^5/well), derived from rhesus macaques, stimulator peptide (10 ng/ml) and competitor peptide (final concentration: 0.1-, 1.0-, 10-, 100- and 1,000-fold relative to the stimulator peptide p3-13). Toxicity of competitor peptides, for either T cells or APC, was checked by mixing either T cells (10^6/well) and 10% IL-2 (Lymphocult-T; Biotech, Frankfurt, Germany) or PBMC (5 × 10^5/well) and 0.5% PHA (Wellcome Diagnostics, Dartford, UK) with competitor peptide (final concentration, 10 μg/ml). To exclude the possibility that the nonresponsiveness to the competitor peptides was due to toxicity of those peptides for either the T cells or the APC, we tested the influence of these peptides on both the IL-2-dependent activation of the HLA-DR17-restricted T cells and the PHA-induced proliferation of the APC. The presence of the competitor peptides did not result in reduction of proliferation of the T cells nor did it disturb the activation by PHA.

Nonhuman Primate Mhc Nomenclature and Cells. The chimpanzee and rhesus macaque Mhc’s have recently been designated MhcPatr and MhcMamu, respectively (23). Official loci and names of chimpanzee and rhesus macaque Mhc class II alleles were given according to the rules formulated by the nonhuman primate Mhc nomenclature committee and were based on similarities between HLA-DRB (human), Patr-DRB (chimpanzee) (20), and Mamu-DRB (rhesus macaque) (21). The Mamu-DR specificities depicted in Fig. 1 were determined by serologic and RFLP techniques. For example, the DR1 specificity was determined by serology, and the A, B, C, and D designations that may follow represent subtypes defined by RFLP (24). All antisera that recognize Patr-DR molecules are at present not available. The Patr-DR types of various chimpanzees that are shown in Fig. 1 have been determined by RFLP (25). There is no obvious relationship between serologic HLA-DR specificities and serologically or RFLP-determined Patr- or Mamu-DR types, meaning that the nonhuman primate DR specificities have been named arbitrarily.

The human homozygous typing EBV-BLCL used in this study are: OOS (HLA-DR1/Dw1), HAR (HLA-DR17, DR52a), and AVL (HLA-DR17, DR52a). The following chimpanzee cells have been used and corresponding relevant sequence information (if present) and typings are depicted: Yvonne (Patr-DRB1*0204, Patr-DRB1*0307/DR4,5), Gwen (Patr-DRB1*0305/DR10), Louise (Patr-DRB1*0201/DR4), Debbie (Patr-DRB1*0701/DR9,10), Annette (Patr-DRB1*0201, Patr-DRB1*0309/DR2,8), Pearl (Patr-DRB1*0203, Patr-DRB1*0306/DR4,7), Wodka (Patr-DRB1*0201, Patr-DRB1*0102/DR14), Sherry (Patr-DRB1*0101/DR3,4), Dennis (Patr-DRB1*0204/DR4B,4C), Brigitte (Patr-DRB1*0102, Patr-DRB1*0702, Patr-DRB1*09/DR9,12), Victoria (Patr-DRB1*0308/DR13). EBV-BLCL of the next rhesus macaques, homozygous for Mamu-A, -B, and -DR, were derived from the Institute of Applied Radiobiology and Immunology–TNO colony: 1WM (Mamu-DRB1*0303/DR1A), JY (Patr-DRB1*0405/DR1B), IRK (not sequenced/DR2), 3C (Patr-DRB1*0309/DR3A), 1KM (Patr-DRB1*0306/DR3C), 2Y (not
sequenced/DR4), 1MC (DRB1*0404/DR5C,5D), 3081 (not sequenced/DR6), 2849 (DRBw8*001/DR7A), 2B (DRB1*0310/DR8), 1ZA (DRB1*0305/DR9).

Results

Binding of hsp65 p3-13 and Hemagglutinin (HA) p307-319 to EBV-BLCL Derived from Chimpanzees and Rhesus Macaques. Hsp65 p3-13 of Mycobacterium leprae and M. tuberculosis functions as an important T cell epitope only in HLA-DR17-positive individuals (11), since it selectively binds to (a pocket in) DR17 molecules (12). It is anticipated that the presence of certain polymorphic amino acid residues (motifs) in the highly polymorphic DRB1 chain, situated in the MHC class II peptide binding site, is required for binding of p3-13 (13). To measure the binding of p3-13 to different Mamu-DR and Patr-DR molecules, 11 different DR homozygous rhesus macaque and 11 disparate chimpanzee EBV-BLCL, selected for distinct DR specificities, were pulsed with biotinylated hsp65 p3-13. As a control for binding, the biotinylated analogue of the influenza HA peptide p307-319 (PKYVKQNTLKLAT) was used. This peptide is recognized by a HLA-DR1-restricted T cell clone (26) and is known to bind well to most or all HLA-DR molecules other than HLA-DR17 (22).

The results of the binding experiments with the nonhuman primate BLCL are shown in Fig. 1. For reference purposes the data for HLA-DR17-positive and -DR1-positive BLCL, good binders for p3-13 and HA p307-319, respectively (12), are also shown. For the chimpanzee-derived BLCL, binding of p3-13 is only found for cells with the Patr-DR13 specificity, whereas none of the other cell lines reach significant levels of binding (Fig. 1A). P3-13 binds to 3 of the 11 rhesus macaque cells tested with the specificities Mamu-DR1, -DR3C, and DR9A. More detailed information on the MHC typing of various chimpanzees and rhesus macaques is given in Materials and Methods. The results represent the mean of three independent experiments.
and -DR9A (Fig. 1 B). In contrast, HA p307-319 binds to all nonhuman EBV-BLCL tested, although the level of fluorescence varies (Fig. 1, C and D).

Comparison of Mhc-DRB1 Sequences of Human, Chimpanzee, and Rhesus Macaque. Since the polymorphic amino acid residues of MHC-DRB molecules involved in binding peptides are likely to be located in the hypervariable regions of the DRβ1 chain (4), the amino acid sequences of the second exons of relevant HLA-, Patr-, and Mamu-DRB gene products were aligned (Fig. 2). One would expect that the various p3-13 binding MHC-DRB chains share specific amino acid residues important for binding. As can be seen, all DRβ chains capable of binding p3-13 (Patr-DRB1*0308, Mamu-DRB*0303, -DRB1*0303, -DRB1*0305, and -DRB1*0306) share two motifs namely at positions 9-13 (EYSTS) and 26-31 (YLYF), which therefore seem to be important for binding (Fig. 2). These important motifs for p3-13 binding have been indicated in Fig. 3, depicting the hypothetical antigen-binding site of MHC class II molecules.

A single mutation, 26Y→F, as seen in Patr-DRB1*0302, -0305, -0306, 0307, and -0309 abrogates binding of p3-13 completely. These data extend the observation on the substitutions of 26Y→F and 28D→E, which are the only differences in the second exon of HLA-DRB1*0301 (HLA-DR17) vs. HLA-DRB1*0302 (HLA-DR18) in humans and abrogates binding of p3-13 as well (Fig. 2, and reference 12).

Various differences between the primate MHC-DRB molecules that are able to bind p3-13, located at positions 32 (H→Y), 37 (N→Y), 47 (F→Y), 57 (D→V,H), 60 (Y→S), 61 (W→Y), 67 (L→Y), 68 (L→V), 70 (Q→D), 71 (K→E,Q), 73 (G→A), 74 (K→A,S), 77 (N→T), 78 (Y→F), 84 (G→R), and 86 (V→G,A,C,F), do not significantly influence peptide binding. Most of these residues do not map within the two motifs important for binding of this particular peptide (4), as can be seen in Fig. 3.

Figure 2. Alignment of the deduced amino acid sequences of relevant HLA-, Patr-, and Mamu-DRB second exon sequences (20, 21, 51). Sequences were obtained from references 20 and 21. As a consensus sequence the HLA-DRB1*0301 allele is used. Differences with this consensus are given by letter substitutions, whereas dashes refer to identical amino acid residues. The regions that are important for p3-13 binding have been boxed, whereas the motifs important for binding are printed in bold.

Figure 3. Schematic representation of the hypothetical model of the antigen-binding site of MHC class II molecules, showing the important conserved (black) and variant (white) residues involved in p3-13 binding. Circles represent amino acid residues that lie in the β-sheet; squares represent amino acid residues that are part of the α-helical structure of the molecule.
Specific Activation of HLA-DR17-restricted T Cells by p3-13 Presented by MHC-DR17-like Nonhuman Primate-derived BLCL. The results above indicate that the two motifs for p3-13 binding have been conserved in evolution within at least part of the Mhc-DRB1*03 lineage members. Mhc-peptide complexes are recognized by T cells only in the context of self-MHC, a phenomenon known as MHC restriction (27). In this light it was investigated whether, apart from the critical peptide-binding residues, polymorphic residues for T cell recognition had also been conserved in evolution. In that case, one would expect that T cells that are selected to see p3-13 in the context of HLA-DR17 may recognize this peptide presented by some nonhuman equivalents that bind the peptide. To test this hypothesis the capacity of the BLCLs of 1WM (Mamu-DRB1*0303), 1KM (Mamu-DRB1*0306), 1ZA (Mamu-DRB1*0305), and Victoria (Patr-DRB1*0308), which all bind p3-13, to present the peptide to a HLA-DR17-restricted, p3-13-reactive T cell clone, CAAp15 1-1, was analyzed in a T cell proliferation assay (Fig. 4). As a positive control human PBMC (DR2,3) were used as APC. All APC were tested for presentation of hsp65 p418-427 to the hsp65-reactive, DR2-restricted T cell clone K2F10 (28). As a negative control rhesus macaque BLCL did not activate the T cells. None of the APC tested, except for the heterozygous human PBMC (DR2,3), were able to present the DR2-specific peptide, hsp65 p418-427, to the HLA-DR2-restricted human T cell clone R2F10.

Inhibition of p3-13-mediated T Cell Activation of a HLA-DR17-restricted T Cell Line by mAbs. To confirm the specificity of the binding of p3-13 to the rhesus macaque DR molecules, we coinoculated p3-13 with either α-DR, α-DP, α-DQ, α-DR52, α-class I, or α-class II mAbs in T cell proliferation assays using either HLA-DR17-positive or Mamu-DR-positive EBV-BLCL as APC (Fig. 5). Here, it is evident that binding of p3-13 is inhibited only by α-DR, α-DR52, and α-class II backbone mAbs. Thus, as expected, the presentation of p3-13 is HLA-DRB1*03 restricted. The fact that the binding is also blocked by α-DR52 seems to be unexpected since 7.3.19.1 reacts with residue 73 (Gly) of DRB1, which is present on some Mhc-DRB1*03 and -DRB3 molecules (29). Examination of the Mamu-DRB1*0305 allele, however, shows the absence of the epitope for 7.3.19.1 binding (Fig. 2). Thus, in this particular case, T cell proliferation is probably inhibited by steric hindrance because mAbs 7.3.19.1 binds to the MHC class II molecules of the human T cell, which does express the epitope in question.

Competition Experiments. Peptides with single amino acid substitutions, which are able to bind to MHC molecules, may not activate T cells (30-32). Such competitor peptides of hsp65, p4-13, have been demonstrated to inhibit the HLA-DR17-restricted T cell response of p3-13-reactive T cell clones (33). These peptides were now tested for their ability to inhibit the response of T cell clone CAAp15 1-1 to p3-13 in the context of rhesus macaque-derived PBMC. The results of the competition experiment are shown in Fig. 6. P4-13 substituted at position 6 (A→Q) was able to inhibit the response induced by p3-13. A control peptide, p4-13 (8D→P), cules have not remained the same. As expected, the nonbinding BLCL did not activate the T cells. None of the APC tested, except for the heterozygous human PBMC (DR2,3), were able to present the DR2-specific peptide, hsp65 p418-427, to the HLA-DR2-restricted human T cell clone R2F10.

Figure 4. Effect on T cell proliferation of the hsp65 p3-13-reactive, DR17-restricted clone, CAAp15 1-1 (A), and the hsp65 p418-427 (LQAAPALDKL)-reactive, DR2-restricted clone, R2F10 (28) (B). Peptides are presented by four different APC. Proliferation assays were performed as described in Materials and Methods. The APC used, given on the x-axis, are human heterozygous PBMC named Leen (HLA-DR2,3) and rhesus macaque-derived EBV-BLCL 1KM (Mamu-DR3C), 1ZA (-DR3), 1WM (-DR1), 1MC (-DR5) and 2849 (-DR7).
which does not bind to HLA-DR17, was not able to inhibit
the T cell response at all.

Discussion

Humans, chimpanzees, and rhesus macaques are primate
species that share a progenitor that lived ~30 million yr ago
(34). The divergence between humans and chimpanzees took
place ~5-7 million yr ago (35). Consequently, these closely
related species may share highly similar immune systems.

Sequencing studies have demonstrated that many MHC poly-
morphisms predate speciation, supporting the trans-species
hypothesis (18), and indicating that some of the polymor-
phisms are relatively old and represent stable structures. The
finding that MHC class II polymorphism is maintained by
strong selective forces (36-39) implies that polymorphism
must have some important function. The biologic signficance
of polymorphism observed within Mhc class II lineages was
investigated by Mhc-peptide binding and T cell proliferation
assays.

Here it is demonstrated that humans, chimpanzees, and
rhesus macaques have particular Mhc-DRB1*03 lineage
members in common. Only Mhc-DRB1*03 molecules that con-
tain residues 9-13 (EYSTS) and 26-31 (YLDRYF) are able
to bind p3-13. This indicates that at least two motifs are neces-
sary for effective p3-13 binding (Fig. 2). As can be seen, the
absence of one of these motifs at 9-13 (EYSTS), as found
in HLA-DRB3*0101, diminished p3-13 binding significantly.
Apart from other residues, a critical residue for p3-13 binding
is 26 (Y), as the substitutions at this position (Y→F) abrogates
p3-13 binding (Fig. 2). The presence of residue 26 (Y), how-
ever, is, on its own, not enough for p3-13 binding as is demon-
strated by the lack of p3-13 binding for HLA-DRB1*0901,
Patr-DRB1*0102, Mamu-DRB1*0402, and -DRB3*0401.

The positively charged residues 71 (K) and 74 (R) are not
present in other HLA alleles besides HLA-DRB1*03, and are
supposed to be located in the peptide-binding site (4). There-
fore, it was proposed that these residues could contribute to
p3-13 binding by interacting with the negative charge on the
peptide (12). However, by using nonhuman primate BLCL,
we now demonstrate that p3-13 binding can occur also to
Mhc alleles Patr-DRB1*0308 and Mamu-DRB1*0303, which
lack positively charged residues such as 71 (K) and 74 (R).

MHC molecules bearing the combination of 9-13 (EYSTS)
and 26–31 (YLDRYF) motifs described above are present in
human, chimpanzee, and rhesus macaque populations. This
indicates that they may have been selected as polymorphic
peptide contact residues (motifs) in the population in order
to preserve binding of certain epitopes from important patho-
gens. Here one such combination is described. However, we
envisage that other preferential MHC-peptide combinations
may have been conserved within distinct primate species as
well.

The reason for this interspecies conservation of class II motifs
may have resulted from the fact that these related species can
share the same pathogenic threats like mycobacterial infec-
tions (40). Thus, some Mhc-DRB lineages, present in different
primates, might have been maintained due to a selective ad-
vantage with regard to diseases, as described for HLA-B53
and severe malaria in West Africa (41). This may also be the
case for mycobacterial diseases, notably tuberculosis cases that
have been described in nonhuman primates (42). In addition,

it has been reported that both chimpanzees (43) and rhesus
macaques (44) can develop leprosy in experimental models,
but even cases of naturally acquired leprosy in chimpanzees
have been documented (45). These examples emphasize that
distinct but closely related species may suffer from shared
pathogenic threats and diseases. Mycobacteria are important
pathogens, and immunity against these pathogens is strictly
T cell dependent both in healthy and in mycobacterial dis-
References

1. Unanue, E.R. 1984. Antigen-presenting function of the macrophage. Annu. Rev. Immunol. 124:533.
2. Schwartz, R.H. 1985. T-lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. Annu. Rev. Immunol. 3:237.
3. Buus, S., A. Sette, and H.M. Grey. 1987. The interaction be-

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between protein-derived immunogenic peptides and Ia. *Immunol. Rev.* 98:115.

4. Brown, J.H., T. Jardetzky, M.A. Saper, B. Samraoui, P.J. Björkman, and D.C. Wiley. 1988. A hypothetical model of the foreign antigen binding site of the class II histocompatibility molecules. *Nature (Lond.)* 332:845.

5. Björkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennet, J.L. Strominger, and D.C. Wiley. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature (Lond.)* 329:506.

6. Young, D.B., R. Lathigra, R. Hendrix, D. Sweetser, and R.A. Young. 1988. Stress proteins are immune targets in leprosy and tuberculosis. *Proc. Natl. Acad. Sci. USA.* 85:4267.

7. Ottenhoff, T.H.M., B. Kale Ab, J.D.A. van Embden, J.E.R. Thole, and R. Kiesling. 1988. The recombinant 65-kD heat shock protein of *Mycobacterium bovis* bacillus Calmette-Guerin/M. tuberculosis is a target molecule for CD4⁺ cytokotic T lymphocytes that lyse human monocytes. *J. Exp. Med.* 168:1947.

8. Ottenhoff, T.H.M., J.B.A.G. Haanen, A. Geluk, T. Mutis, B. Kale Ab, J.E.R. Thole, W.C.A. van Schooten, P.J. van der Elsen, and R.R.P. de Vries. 1991. Regulation of Mycobacterial heat shock protein-reactive T cells by HLA class II molecules: lessons from leprosy. *Immunol. Rev.* 121:171.

9. Lamb, J.R., V. Bal, P. Mendez-Sempario, A. Mehler, A. So, J. Rothbard, S. Jindal, R.A. Young, and D.B. Young. 1989. Stress proteins may provide a link between the immune response to infection and autoimmunity. *Int. Immunol.* 1:191.

10. Thole, J.E.R., W.J. Keulen, A.H.J. Kolk, D.G. Groothuis, L.G. Berwald, R.H. Tiesjema, and J.D.A. van Embden. 1987. Characterization, sequence determination and immunogenicity of a 64-kilodalton protein of *Mycobacterium bovis* BCG expressed in *Escherichia coli* K12. *Infect. Immun.* 55:1466.

11. Van Schooten, W.C.A., D.G. Ellerink, J. Van Embden, D.C. Anderson, and R.R.P. de Vries. 1989. DR3-restricted T cells from different HLA-DR3-positive individuals recognize the same peptide (amino acids 2-12) of the mycobacterial 65-kDa heat-shock protein. *Eur. J. Immunol.* 19:2075.

12. Geluk, A., W. Bloemberhof, R.R.P. De Vries, and T.H.M. Ottenhoff. 1992. Binding of a major T cell epitope of mycobacteria to a specific pocket within HLA-DRw17(DR3) molecules. *Eur. J. Immunol.* 22:107.

13. Lawlor, D.A., F.E. Ward, P.D. Ennis, A.P. Jackson, and P. Parham. 1988. HLA-A and B polymorphisms predate the divergence of humans and chimpanzees. *Nature (Lond.)* 353:268.

14. Mayer, W.E., M. Jonker, D. Klein, P. Ivanyi, G. Seventer, and J. Klein. 1988. Nucleotide sequences of chimpanzee MHC class I alleles: evidence for transspecies evolution of *EMBO (Eur. Mol. Biol. Organ.)* J. 7:2765.

15. Watkins, D.I., Z.W. Chen, A.L. Hughes, M.G. Evans, T.F. Tedder, and N.L. Letvin. 1991. Evolution of the MHC class I genes of a New World primate from ancestral homologues of human non-classical genes. *Nature (Lond.)* 346:60.

16. Fan, W., M. Kasahara, J. Gutknecht, D. Klein, W.E. Mayer, M. Jonker, and J. Klein. 1989. Shared class II MHC polymorphism between humans and chimpanzees. *Hum. Immunol.* 26:107.

17. Gyllensten, U.B., M. Sundvall, I. Eczcusa, and H.A. Erlich. 1991. Genetic diversity at class II DRB loci of the primate MHC. *J. Immunol.* 146:4368.

18. Klein, J. 1987. Origin of major histocompatibility complex polymorphisms: the transspecies hypothesis. *Hum. Immunol.* 19:155.

19. Otting, N., M. Kenter, P. van Weeren, M. Jonker, and R.E. Bontrop. 1992. Mhc-DQB repertoire variation in hominoid and Old World primate species. *J. Immunol.* 149:461.

20. Kenter, M., N. Otting, J. Anholt, M. Jonker, R. Schipper, and R.E. Bontrop. 1992. Evolutionary stability of trans-species major histocompatibility complex class II DRB lineages in man and rhesus monkey. *Hum. Immunol.* 35:29.

21. Slierendregt, B.L., J.T. van Noort, R.M. Bakas, N. Otting, M. Jonker, and R.E. Bontrop. 1992. Evolutionary stability of trans-species major histocompatibility complex class II DRB lineages in man and rhesus monkey. *Hum. Immunol.* 35:29.

22. Busch, R., G. Strang, K. Howland, and J.B. Rothbard. 1990. Degenerate binding of immunogenic peptides to HLA-DR proteins on B cell surfaces. *Int. Immunol.* 2:443.

23. Klein, J., R.E. Bontrop, R.L. DAWinns, H.A. Erlich, U.B. Gyllestuen, E.R. Heise, P.P. Jones, P. Parham, E.K. Wakeland, and D.I. Watkins. 1990. Nomenclature for the major histocompatibility complex of different species: a proposal. *Immunogenetics.* 31:217.

24. Slierendregt, B.L., N. Otting, M. Jonker, and R.E. Bontrop. 1991. RFLP analysis of the rhesus monkey Mhc class II DR subregion. *Hum. Immunol.* 30:11.

25. Bontrop, R.E., L.A.M. Broos, K. Pham, R.M. Bakas, N. Otting, and M. Jonker. 1990. The chimpanzee major histocompatibility complex class II DR subregion contains an unexpectedly high number of beta-chain genes. *Immunogenetics.* 32:272.

26. Rothbard, J.B., R. Busch, K. Howland, V. Bal, C. Fenton, W.R. Taylor, and J.R. Lamb. 1989. Structural analysis of a peptide-HLA class II complex: identification of critical interactions for its formation and recognition by T cell receptor. *Int. Immunol.* 1:479.

27. Zinkernagel, R.M., and P.C. Doherty. 1974. Restriction of intracellular tyrosinase in lymphocytic choriomeningitis within a syngeneic or allogeneic system. *Nature (Lond.)* 284:701.

28. Anderson, D.C., W.C.A. Van Schooten, A.A.M. Janson, M.E. Barry, and R.R.P. De Vries. 1990. Molecular mapping of interactions between a *Mycobacterium leprae*-specific T cell epitope, the restricting HLA-DR2 molecule, and two specific T cell receptors. *J. Immunol.* 144:2459.

29. Bontrop, R.E., D.G. Ellerink, N. Otting, M. Jonker, and R.R.P. de Vries. 1990. Major histocompatibility complex class II-restricted antigen presentation across a species barrier: conservation of restriction determinants in evolution. *J. Exp. Med.* 172:53.

30. Werdle, O. 1982. Chemically related antigens compete for presentation by accessory cells to T cells. *J. Immunol.* 129:1883.

31. Rock, K.L., and D.G. Groothuis. 1983. Inhibition of antigen-specific T lymphocyte activation by structurally related Ia gene-controlled polymers. Evidence of specific competition for accessory cell antigen presentation. *J. Exp. Med.* 157:1618.

32. Babbitt, B.P., G. Matsueda, E. Haber, E.R. Unanue, and P.M. Allen. 1986. Antigenic competition at the level of peptide-Ia binding. *Proc. Natl. Acad. Sci. USA.* 83:4509.

33. Geluk, A., K.E. van Meijgaard, A.A.M. Janson, R.H. Meloen, J. Drijfhout, R.R.P. de Vries, and T.H.M. Ottenhoff. 1992. Functional analysis of DR17(DR3)-restricted mycobacterium T cell epitopes reveals DR17 binding motif and enables the design of allele specific competitor peptides. *J. Immunol.* 149:2864.

34. Miyamoto, M.M., B.F. Koop, J.L. Slightom, M. Goodman, and M.R. Tennant. 1988. Molecular systematics of higher primates: genealogical relations and classifications. *Proc. Natl. Acad.*
35. Andrews, P. 1986. Fossil evidence on human origins and dispersal. Cold Spring Harbor Symp. Quant. Biol. 51:419.
36. Benoist, C.O., D.J. Mathis, M.R. Kanter, V.E. Williams, and H.O. Mc Devitt. 1983. Regions of allelic hypervariability in the murine Ac immune response gene. Cell. 34:169.
37. Hughes, A.L., and M. Nei. 1989. Nucleotide substitution at major histocompatibility complex class II loci: evidence for overdominant selection. Proc. Natl. Acad. Sci. USA. 86:958.
38. Serjeantson, S.W. 1989. The reasons for MHC polymorphism in man. Transplant. Proc. 21:598.
39. Erlich, H.A., and U.B. Gyllensten. 1991. The evolution of allelic diversity at the primate major histocompatibility complex class II loci. Hum. Immunol. 30:110.
40. Klein, J. 1991. Of HLA, tryps, and selection: an essay on coevolution of MHC and parasites. Hum. Immunol. 30:247.
41. Hill, A.V.S., C.E.M. Allsopp, D. Kwiatkowski, N.H. Anstey, P. Twumasi, P.A. Rowe, S. Bennett, D. Brewster, A.J. McMichael, and B.M. Greenwood. 1991. Common West African antigens are associated with protection from severe malaria. Nature (Lond.). 352:595.
42. Mulder, J.B. 1976. Tuberculosis in non-human primates. Vet. Med. Small Anim. Clin. 71:1286.
43. Gunders, A.E. 1958. Progressive experimental infection with Mycobacterium leprae in a chimpanzee. A preliminary report. J. Trop. Med. Hyg. 61:228.
44. Wolf, R.H., B.J. Gormus, L.N. Martin, G.B. Baskin, G.P. Walsh, W.M. Meyers, and C.H. Binford. 1985. Experimental leprosy in three species of monkeys. Science (Wash. DC). 227:529.
45. Leiniger, J.R., K.J. Donham, and M.J. Rubino. 1978. Leprosy in chimpanzee. Morphology of the skin lesions and characterization of the organism. Vet. Pathol. 15:339.
46. Sigurdardottir, S., C. Borsch, K. Gustafsson, and L. Andersson. 1992. Exon encoding the antigen-binding site of MHC class II β-chains is divided into two subregions with different evolutionary histories. J. Immunol. 148:968.
47. Belich, M.P., J.A. Madrigal, W.H. Hildebrand, J. Zemmour, R.C. Williams, R. Luz, M.L. Petzi-Erler, and P. Parham. 1992. Unusual HLA-B alleles in two tribes of Brazilian Indians. Nature (Lond.). 357:326.
48. D.L. Watkins, S.N. McAdam, X. Liu, C.R. Strang, E.L. Milford, C.G. Levine, T.L. Garber, A.L. Dogon, C.I. Lord, S.H. Ghim, G.M. Troup, A.L. Hughes, and N.L. Letvin. 1992. New recombinant HLA-B alleles in a tribe of South American Amerindians indicate rapid evolution of class I loci. Nature (Lond.). 357:329.
49. Levinson, G., A.L. Hughes, and N.L. Letvin. 1992. Sequence and diversity of rhesus monkey T cell receptor β chain genes. Immunogenetics. 35:75.
50. Tiwari, J.L., and P.I. Terasaki. 1985. HLA and Disease Associations. Springer-Verlag New York Inc., New York, 195 pp.
51. Marsh, S.G.E., and J.G. Bodmer. 1991. HLA Class II nucleotide sequences. Hum. Immunol. 31:207.