because the onset and progression of the disease is markedly delayed.

Taken together, these results demonstrate that IL-4 is critical to the development of MAIDS. The mechanism by which IL-4 mediates its effects in the development of the disease is not clear. Infection with the LP-BM5 virus causes a switch from a Th1 to a Th2 response with progression of the disease (*2*). This may be due to superantigen activity expressed by virus-infected B cells (*10*) that stimulate aberrant IL-4 production in T cells. A mouse line that expresses high amounts of IL-4 derived from a transgene shows a variety of immunological abnormalities, including a deficient proliferative response of T cells to the mitogen concanavalin A (*11*). Furthermore, Th2 cytokine responses are dependent on IL-4 production (*12*). Thus, other Th2 cytokines that have been shown to be significantly reduced in IL-$4^{-/-}$ mice, such as IL-5, IL-9, IL-10, and IL-6 (*13*), may also be involved in the development of MAIDS. This could be tested by reconstituting infected IL-$4^{-/-}$ mice or by infecting mice deficient in other cytokines. Although human immunodeficiency virus (HIV) and the murine leukemia virus causing MAIDS represent different types of retroviruses, some of the features of the diseases are similar, such as lymphoadenopathy, B cell hyperactivity, T cell immunodeficiency, and development of certain tumor cells at the late stage of the disease (*1*, *14*). Furthermore, a shift from a Th1 to a Th2 response and a high serum immunoglobulin E level have been reported for HIV-infected patients (*15*). These findings suggest that IL-4 as well as other Th2-related cytokines may be crucial to the development of T cell dysfunction induced by the two different viruses. Thus, understanding the role of IL-4 in the development of MAIDS may provide insight into the mechanisms causing dysregulated cytokine production and generalized T cell immunodeficiency found in human acquired immunodeficiency syndrome.

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HLA-DPB1 Glutamate 69: A Genetic Marker of Beryllium Disease

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Chronic beryllium disease (CBD) is a lung disorder related to beryllium exposure and is characterized by the accumulation in the lung of beryllium-specific $CD4^+$ major histocompatibility complex (MHC) class II–restricted T lymphocytes. Evaluation of MHC class II genes in 33 CBD cases and 44 controls has shown a negative association with HLA-DPB1*0401 ($P < 0.001$) and a positive association with HLA-DPB1*0201 ($P < 0.05$) alleles, which differ at residues 36, 55 to 56, and 69 of the β_1 chain. Among CBD cases, 97 percent expressed the HLA-DPB1*0201–associated glutamic acid (unaffected population, 30 percent; $P < 0.001$) at residue 69, a position involved in susceptibility to autoimmune disorders. This suggests that HLA-DP has a role in conferring susceptibility and that residue 69 of HLA-DPB1 could be used in risk assessment for CBD.

Exposure to metals such as Co, Al, Ti, Zr, and Be is associated with a variety of chronic disorders of the lung (*1*). CBD is a hypersensitivity lung disorder caused by exposure to Be that is not strictly dependent on Be concentration (*2*). As predicted by its immunopathologic features, CBD is maintained by an accumulation of large numbers of Be-specific $CD4^+$ T cells in patient lungs (*3*). In this context, CBD could be used as a model to test the relation between environmental and immunogenetic factors in occupation-related diseases. T cell clones from CBD patient lungs are MHC class II–restricted, that is, they only respond to Be in association with MHC class II molecules on the surface of the antigen-presenting cell (*3*). Genes of the MHC class II (HLA-DR, -DQ, and -DP) that are associated with susceptibility to autoimmune disorders (*4*) are likely candidates as susceptibility genes to CBD. This hypothesis is supported by the observations that MHC class II genes are involved in T cell responses to metals such as Ni and Au (*5*), and they play a key role in susceptibility to experimental hypersensitivity to Be and other metals (*6*).

We tested this concept, after a preliminary analysis of a small sample of patients that did not show strong associations with HLA-DR or HLA-DQ genes, by evaluating the association of the HLA-DP gene with CBD in a group of 33 CBD individuals and a group of 44 Be-exposed unaffected individuals (*7*). Frequencies of the HLA-DPB1 alleles in the Be-exposed unaffected group were similar to those of the normal population (*8*), regardless of the race composition of the group. The HLA-DPB1*0201 and *0401 allele frequencies in the total population sample were similar to those in Caucasians alone [percent of total population sample unaffected, *0201 (10%), *0401 (48%); Caucasians unaffected, *0201 (11%), *0401 (52%); $P > 0.2$, compared with published populations (8)] (9). In contrast, allelic frequencies in the CBD case group were biased. The frequency of the DPB1*0201 allele was increased [CBD, 30%; unaffected, 10%; $\chi^2 = 9.94$, $P = 0.0016$, Bonferroni corrected $P < 0.05$ (19 alleles)]. Conversely, the frequency of the DPB1*0401 allele was decreased (CBD, 14%; unaffected, 48%; $\chi^2 = 19.79$, $P = 0.00001$, corrected $P < 0.001$). When we evaluated the phenotypic frequencies, we observed a similar bias (DPB1*0201: CBD, 52%; unaffected, 18%; corrected $P < 0.05$; DPB1*0401: CBD, 27%; unaffected, 68%; corrected $P < 0.01$), indicating that, as in

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other human leukocyte antigen (HLA)–associated diseases, the expression of at least one disease-associated HLA allele is sufficient to confer susceptibility.

The HLA-DPB1 gene comprises six hypervariable coding regions, denoted as A, B, C, D, E, and F (*10*). The DPB1*0201 and DPB1*0401 alleles differ in three of these regions: DPB1*0201 codes Val for Ala at position 36 (B), Asp Glu for Ala Ala at positions 55 and 56 (C), and Ile Leu Glu Glu Glu for Ile Leu Glu Glu Lys at positions 65 to 69 (D). Although no significant association existed for region B, evaluation of region C showed significant association with disease (79% of the CBD group compared with 41% of the unaffected group; corrected $P < 0.005$). An even stronger association was shown for region D (CBD, 97%; unaffected, 27%; corrected $P < 0.0001$) (Fig. 1).

We confirmed this association by a DNA amplification assay (amplification refractory mutation system, ARMS) with primers specific for either Glu^{69} or Lys^{69} coding sequences (the sequences present on the DPB1*0201 and *0401 alleles, respectively) and direct sequencing of the HLA-DPB1 polymerase chain reaction (PCR)–amplified DNA. The frequency of the Glu^{69} phenotype was higher in the CBD group compared with the unaffected group (Fig. 2). The Glu^{69} variant showed a strong association with CBD (corrected $P < 0.0002$, P corrected for 19 HLA-DPB1 region, single amino acid–coding variants). The data suggest an association of CBD with the HLA-DPB1 Glu^{69} epitope.

To explore the possibility that this association may be due to linkage disequilibrium with more telomeric genes in the MHC locus, we analyzed the association of Glu^{69} with other genes in the MHC locus in CBD patients. Two markers were investigated: HLA-DRB4*0101, which is expressed by ~60% of the Caucasian population and is indirectly associated with rheumatoid arthritis (*11*), and TNFB*1, which is associated with the B8, DR3 haplotype and autoimmunity (*12*). No association with CBD was found for HLA-DRB4*0101 [CBD, 54%; unaffected, 61%; $P > 0.9$ (*13*)]. Similarly, no association with CBD was found for the 5.3-kb Nco I TNFB*1 allele [CBD, 8.3%; unaffected, 6.0%; $P > 0.7$ (*14*)].

Complete genotyping of the MHC locus may be required to formally rule out the possibility that linkage to other MHC genes influences the association of HLA-DPB1 Glu^{69} with CBD. However, our observations suggest that HLA-DP could be the primary genetic factor of susceptibility to CBD. Furthermore, analysis of the relation of MHC class II molecule structure and function suggests that residues 55 and 69 may be directly implicated in the response to Be. Residue 55 is involved in formation of a salt bridge with an Arg residue at position 84 of the α chain that is critical to the conformation of the antigen binding pocket. Residue 69 is a negatively charged residue (*15*) that may directly interact with Be^{2+} itself. Studies on the association of MHC class II with chronic autoimmune disorders have shown that single amino acid changes at positions 57 and 71 of HLA-DQB1 and HLA-DRB1 chains [corresponding to positions 55 and 69 on the HLA-DPB1 chain (*15*)] are associated with susceptibility to disorders such as insulin-dependent diabetes mellitus and rheumatoid arthritis (*16*). Similarly, amino acid changes (at positions 55 to 57 and 69) on the HLA-DPB1 subunit are associated with juvenile rheumatoid arthritis and coeliac disease (*17*). Because the antigens involved in these disorders are not known yet, it is not clear whether these substitutions affect the ability of disease-associated HLA molecules to bind to specific antigens such as in the allergic encephalomyelitis model (*18*) or affect their ability to regulate specific immune responses as in the schistosomiasis model (*19*).

Whatever the mechanism involved, this study implicates HLA-DPB1 Glu^{69} as a Be disease risk marker. CBD is thus a model of environmental hypersensitivity with which to assess the potential value of immunogenetic testing in occupational risk prevention. Although the implementation of the Atomic Energy Commission's 1950 recommendations calling for a 99% reduction of Be concentrations in the work environment completely eliminated acute Be pneumonia, the incidence of CBD was not similarly reduced (*20*). In the context that CBD incidence may reach 15.8% in certain worker groups (*21*), the risk associated with

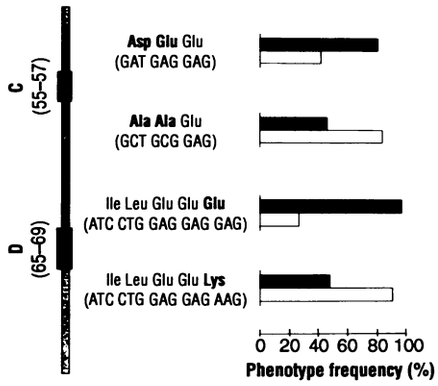

Fig. 1. Expression of HLA-DPB1 polymorphisms coding for amino acid substitutions at variable regions C and D in the CBD case group (solid bars) and the Be-exposed unaffected case group (open bars). (**Left**) Schematic diagram of the C and D polymorphic regions on the HLA-DPB1 chain. (**Right**) We determined the frequencies of C polymorphisms coding for the DPB1*0201-associated Asp-Glu-Glu and DPB1*0401-associated Ala-Ala-Glu variants (positions 55 to 57) and the DPB1*0201-associated Ile-Leu-Glu-Glu-Glu and DPB1*0401-associated Ile-Leu-Glu-Glu-Lys variants (positions 65 to 69). The frequencies were determined by hybridization of PCR-amplified DNA (primers UG19 and UG21) with the ^{32}P-labeled variant-specific oligonucleotide probes DB14 (CAGTACCTCCTCATCAGG) and DB13 (CCTGCTGCGGAGTACTG) for region C and DB19 (GCTCCTCCTCCAAGGATGTC) and DB18 (GACATCCCTGGAGGAGAAGC) for region D, respectively (*10*).

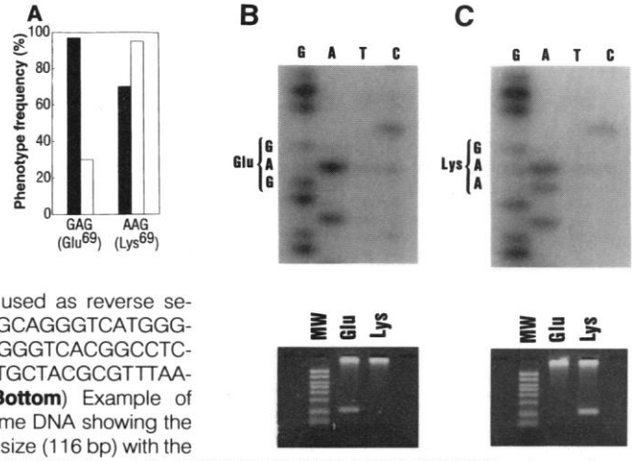

Fig. 2. (**A**) Frequency of the HLA-DPB1 Glu^{69} and Lys^{69} phenotypes in the CBD case (solid bars) and Be-exposed unaffected case (open bars) groups, as determined by ARMS testing (*25*). (**B**) (**Top**) Example of a Glu^{69} PCR-amplified (UG19 and UG21) DNA sample, sequenced by a modification of the Sanger technique (*26*). We used as reverse sequencing primers DP1 (GCTGCAGGGTCATGGGCCCGC) or DP2 (GCTGCAGGGTCACGGCCTCGT) (3′ to 5′) and DPCS1 (TGCTACGCGTTTAATGGGACAC) (5′ to 3′). (**Bottom**) Example of ARMS amplification of the same DNA showing the PCR product of the expected size (116 bp) with the UG21B (AGCCGGCCCAAAGCCCTCA) and Glu^{69} (GAAGGACATCCTGGAGGAGG) primers (lane Glu). No amplification was seen with the UG21B and the Lys^{69} (GAAGGACATCCTGGAGGAGA) primers (lane Lys) (2.5% agarose gel). Lane MW, molecular weight markers (BioLow, BioVentures). (**C**) (**Top**) Lys^{69} PCR-amplified (UG19 and UG21) DNA sequenced as described above. (**Bottom**) ARMS amplification of the same DNA, showing the expected PCR product (116 bp) with the UG21B and Lys^{69} primers (lane Lys) and no amplification with the UG21B and the Glu^{69} primers (lane Glu) (2.5% agarose gel).

the HLA-DPB1 Glu^{69} marker may be high.

Screening for disease-associated genetic markers may provide new tools for prevention of occupational diseases (*22*). So far in CBD, blood and lung lymphocyte testing for Be-specific proliferation have been used to identify individuals with Be allergy and disease (*23*). However, because lymphocyte tests do not predict disease or risk, genetic testing may have a role, in association with lymphocyte Be-specific proliferation testing, in the identification and follow-up of individuals at risk of beryllium disease.

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7. A group of 33 CBD cases (46 ± 16 years of age; 30 males and 3 females; 30 Caucasians, 2 Hispanics, and 1 African American) were retrospectively identified according to established criteria (see below) including (i) a history of exposure; (ii) compatible chest x-ray abnormalities; (iii) abnormal lung function tests [vital capacity (VC) 79 ± 18% of predicted and diffusing capacity (DLCO) 62 ± 21% of predicted]; (iv) a pulmonary biopsy showing noncaseating granulomas; and (v) a positive Be-stimulated lymphocyte proliferation test (LTT) in the blood or bronchoalveolar lavage (the test was performed according to published protocols in each of the contributing institutions) (*2*, *3*). A group of 44 Be-exposed unaffected cases [40 ± 9 years of age; 25 males and 19 females; 31 Caucasians, 11 Hispanics, and 2 African Americans ($P > 0.09$ compared with CBD cases)] were identified out of a group of 46 Be workers during a CBD survey at a Be plant (Brush Wellman Medical Department, Tucson, AZ). None had a history of pulmonary disease or abnormal chest x-rays or pulmonary functions. We identified Be-exposed individuals as cases by LTT tests and clinical evaluation, as described above. CBD cases were evaluated at the Pulmonary Branch of NIH, Bethesda, MD; the Cleveland Clinic, Cleveland, OH; the National Jewish Center for Immunology and Respiratory Medicine, Denver, CO; the University of Pennsylvania, Philadelphia, PA; and the University of Wales, Penarth, United Kingdom. Be-exposed individuals were evaluated at the Brush Wellman Medical Department; blood LTTs were performed at Specialty Laboratories (Santa Monica, CA) and at the National Jewish Center for Immunology and Respiratory Medicine according to standard procedures. Informed consent was obtained from all individuals.
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9. The typing of HLA-DPB1 was carried out by gel electrophoresis heteroduplex analysis (*24*). A 311-bp fragment spanning the entire DPB1 exon 2 was generated by PCR with the primers UG19 (GCTGCAGGAGAGTGGCGCCTCCGCTCAT) and UG21 (CGGATGCCGGCCCAAAGCCCTCACTC) using phenol/chloroform-extracted DNA from snap frozen Ficoll-Hypaque–purified peripheral blood mononuclear cells (*3*). The PCR product was further used as a template to obtain DNA from alleles DPB1*0201, *0202, *0401, *0402, *1501, and *1801 (group 1) and DPB1*0101, *0301, *0501, *0601, *0801, *0901, *1001, *1101, *1301, *1401, *1601, *1701, and *1901 (group 2) with the hypervariable region F-complementary oligonucleotides DP1 and DP2 as the 3′ end primers and the oligonucleotide UG19 as the 5′ end primer. The PCR reactions were carried out with standard protocols. Unclear typing results were confirmed by sequence-specific oligonucleotide DNA hybridization and direct sequencing of the PCR product. All data are presented as means ± SDs. Demographic data were compared by variance analysis. We compared the allele and polymorphism frequencies by the χ^2 test with Bonferroni correction according to which the P values are multiplied by the number of allelic variants tested for each comparison (J. L. Tiwari and P. I. Terasaki, Eds., *HLA and Disease Associations* (Springer-Verlag, New York, 1985), pp. 18–27].
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13. We carried out analysis of the HLA-DRB4*0101 gene frequencies in the CBD case and Be-exposed unaffected case groups with an ARMS assay with the primers DRw53-1 (TCCTCAATGGGACGGAGCGA) and DRw53-2 (CTCCACAACCCCGTAGTTGTA), specifically amplifying a 239-bp DNA fragment.
14. Analysis of the frequencies of the TNFβ Nco I polymorphism was carried out by DNA amplification of the TNFβ gene with the primers Nco I-1 (CCGTGCTTCGTGCTTTGGACTA) and Nco I-2 (AGAGCTGGTGGGGACATGTCTG) followed by Nco I restriction fragment polymorphism analysis of the 740-bp PCR product, as described by Messer *et al.* (*12*).
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Regional Codon Randomization: Defining a TATA-Binding Protein Surface Required for RNA Polymerase III Transcription

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The TATA-binding protein (TBP) is required for transcription by all three nuclear RNA polymerases. TBP was subjected to regional codon randomization, a codon-based mutagenesis method that generates complex yet compact protein libraries. Analysis of 186 temperature-sensitive TBP mutants yielded 65 specifically defective in transcription by RNA polymerase III (Pol III). These mutants map to a limited TBP surface that may interact with Tds4, a component of the Pol III transcription factor TFIIIB. Strains that contain the Pol III–defective derivatives have increased amounts of messenger RNA, which suggests that competition among TBP-interacting factors for limiting quantities of TBP determines the ratio of Pol II and Pol III transcription in vivo.

The TATA-binding protein (TBP) is required for transcription by all three nuclear RNA polymerases (*1*–*4*). TBP interacts with TATA-associated factors (TAFs) to form distinct complexes that are specific for the individual RNA polymerases (Pol). The Pol II–specific complex, TFIID, contains at least 10 TAFs that have been