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Generation of Anti-HLA-DR4 Specific Antibodies by Immunization of the Recombinantly Expressed Allelic Subtype-Specific Region of the HLA-DRB1*0405 Molecules

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HLA-DR4 is the dominant allele of MHC class II genes in Koreans. In particular, the DRB1*0405 subtype has been reported to be almost exclusively expressed in Far East Asians, and has also been observed to be strongly associated with rheumatoid arthritis in Koreans and the Japanese. Identification of this specific allele has been mainly performed by PCR-based methods, which is often time consuming, costly, and involves tedious procedures such as the isolation of genomic DNA, PCR, and gel electrophoresis. To develop a more convenient tool for screening vast amounts of samples as well as to generate reagents which might also be used in other applications, in this study, antibodies were produced against this specific HLA subtype. By PCR, an allele-specific region covering the β1 domain of DRB1*0405 was amplified and recombinantly expressed in *E.coli*. Immunization of Lewis rats with the purified protein yielded an allele specific antiserum. Western blot analysis showed the selective detection of the HLA-DR β-chain. Using this antiserum, established cell lines and peripheral blood lymphocytes were analyzed on their HLA haplotype by fluorescence activated flow cytometry. These novel antibodies will provide a powerful tool in the detection and investigation of DR4 alleles.

Keywords: Allelic typing, Antibodies, HLA-DR, MHC Class II, Recombinant protein.

Introduction

The class II molecules of the major histocompatibility complex are expressed as heterodimeric glycoproteins consisting of a noncovalently associated ~33 kDa α-chain and ~28 kDa β-chain (Kappes and Strominger, 1988). Detected mostly in cells involved in the presentation of foreign antigens to the host immune system, such as B-cells, macrophages, and dendritic cells (Unanue, 1984; Pierce *et al.*, 1988; Steinman, 1991; Cresswell, 1994), the MHC class II molecules are responsible for the activation and stimulation of T-helper cells (Swain *et al.*, 1984), the thymic development of CD4+ T-cells (Blackman *et al.*, 1989) as well as in the binding and presentation of viral and bacterial superantigens (Mollick *et al.*, 1989; Choi *et al.*, 1992; Winslow *et al.*, 1994). Furthermore, the expression of certain alleles of the MHC class II molecules have also been reported in connection with some autoimmune diseases (Matsushita *et al.*, 1994) as well as non- or low-responsiveness to immunization of several preventive vaccines (Alper *et al.*, 1989; Varla-Leftherioti *et al.*, 1990). For these obviously important reasons, the class II molecules of the human have for a long time been the subject of intensive studies. In particular, the relationship of some specific allelic subtypes and the increased susceptibility to certain diseases are well investigated. In the case of the human MHC (human leukocyte antigen, HLA) class II complex, the DRB1*0405 allele had been described to show a significant association with some autoimmune diseases such as the Vogt-Koyanagi-Harada's diseases (Shindo *et al.*, 1994) or rheumatoid arthritis (Wordsworth and Bell, 1992; Kim *et al.*, 1997). The identification of such a close linkage between disease susceptibility and this specific HLA-DRB1*0405 haplotype is of extraordinarily interest, especially for Far East Asians, since this allelic subtype has been repeatedly reported to be dominantly expressed in this population (Imanishi *et al.*, 1992). The ethnic association of certain HLA haplotypes is a well-

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documented fact (Svensson *et al.*, 1996; Gaudieri *et al.*, 1997). It had been reported that HLA-DRB1*0401 is the major DR4 allele in Caucasians, in contrast to HLA-DRB1*0405 which is predominant in Orientals. The identification of the HLA-genotype in humans has been carried out by several alternative methods. The best established might be the serological method, where there are haplotype cross-reactive human sera, defining the so-called "serotype" of HLA alleles. But due to the low resolution, alternative or improved methods have been established enabling a deeper insight into the molecular background behind the serological differences. Among these are methods like the PCR-restriction fragment length polymorphism (PCR-RFLP), PCR-single strand conformation polymorphism (PCR-SSCP), PCR-sequence specific oligonucleotides (PCR-SSO), and DNA heteroduplex generator (DHG), just to name a few (Moribe *et al.*, 1996, Savage *et al.*, 1996;). But, while the information about DNA sequences is quite of importance, it is also true that often pure DNA information alone cannot allocate a given allele into serological clusters, which is still the most important criteria in clinical applications as well as in HLA phylogeny and nomenclature (Gorski, 1989). In so far, serological determination will remain a major factor for the identification of HLA alleles. To facilitate this process, which is also in advantage to the more costly and labor intensive PCR-based methods, in this study, HLA-DR4 specific antisera were developed in the rat. Immunization with recombinantly expressed polypeptides from the most allele specific part of the class II β-chain assured the generation of antibodies which discriminate in high resolution allelic differences within the HLA-DR4 antigens.

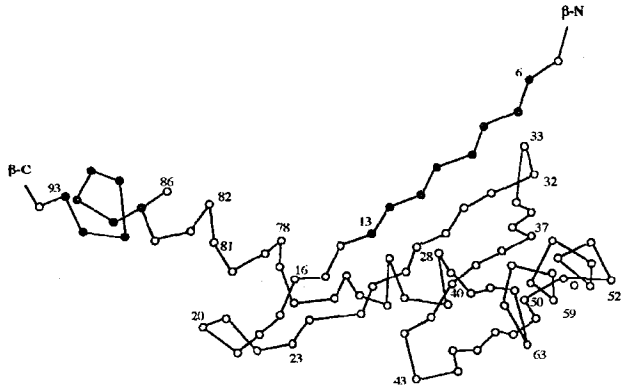

Fig. 1. Molecular structure of the HLA-DRB antigen and the schematic presentation of the recombinantly expressed region within the β1 domain. Closed circles indicate the positions of the PCR primers on amino acid levels which were used for amplification of the allele-specific region. Sequences inbetween have been recombinantly expressed. The structure was adapted from the original drawing of the HLA-DR1 molecules as reported by Stern *et al.* (1994).

MRGSHHHHHH TDPHAPGRHG RGIFLEQVKH

ECHFFNGTER VRFLDRYFYH QEESVRFDSD

VGEYRAVTEL GRPVPSTGTA RRTSWSRGGP

RWTPTADTTT GLVRASQCSE SLVRPPAGRP

AAKL

Fig. 2. Amino acid sequence of the recombinantly expressed protein. Amino acids in bold letters indicate the HLA-DRB1*0405 derived sequences, underlined seqences are derived from the pGEM-T vector, and the remaining flanking sequences are encoded in the pQE prokaryotic expression vector.

Materials and Methods

Subcloning of the allele-specific region of the HLA-DRB1*0405 A 261-bp fragment from the exon-2 region of the HLA-DRB1*0405 was amplified from cDNA of the EBV-transformed B-cell line "Wa" (Maeng *et al.*, 1997) using the following sequence specific primers: upstream primer, 5′-gtttcttggagcaggttaaac-3′; downstream primer, 5′-cgctgcactgtgaagctctc-3′ (Fig. 1). The amplified fragment was cloned into the pGEM-T vector (Promega, Madison, USA) by taking advantage of the protruding adenosine bases on each end of the PCR fragment. The whole inserted fragment was then excised by digestion with the restriction enzymes *Sph*I and *Sal*I and subcloned into the corresponding sites of the pQE-30 prokaryotic expression vector (Qiagen Inc., Chatsworth, USA). Nucleotide sequence analysis of this recombinant plasmid revealed that a single nucleotide deletion had occurred while cloning into the pGEM-T vector which resulted in a frameshift of the subcloned insert. To compensate this missing nucleotide, the whole fragment was excised again, this time by the restriction enzymes *Hind*III and *Bam*HI. This DNA fragment was cloned into the corresponding sites of the pQE-31 plasmid (Qiagen Inc.) resulting in the recombinant expression vector, pQE-Ex2/B1*0405. As confirmed by nucleotide sequence analysis, the open reading frame encoded a 124 a.a. polypeptide (Fig. 2) containing 87 a.a. from the exon-2 region of the HLA-DRB1*0405 cDNA.

Expression and isolation of the recombinant proteins Recombinant *E.coli* of the strain JM109 bearing the pQE-Ex2/B1*0405 plasmid was used for expression of the desired recombinant protein. For one preparation, cells were grown in 100 ml of LB media with 50 μg/ml ampicillin at 37°C, and the cells were induced in their log growth phase by addition of IPTG to a final concentration of 0.3 mM. The cell culture was then incubated for a further 3 h on a shaker platform. After cell harvesting, the pellet was resuspended in 1/20 volume of sonication buffer (50 mM Na-phosphate, pH 8.0, 300 mM NaCl), and the cell wall was disrupted by mild sonication. After centrifugation at 4000 × *g* for 10 min, the supernatant was discarded, and the pellet was resuspended in 1 ml of denaturation buffer (8 M urea, 0.1 M Na-phosphate, 10 mM Tris-HCl, pH 8.0).

The whole sample was loaded on a 12.5% SDS-polyacrylamide gel and separated by gel electrophoresis under reducing conditions. Identification of the induced band was carried out by gel staining in 0.3 M $CuCl_2$ for 10 min under continuous shaking. Recombinant protein could be recovered from the gel fragment by electroelution in dialysis tubing (Spectrum, Houston, USA) with a molecular cutoff size of 6–8 kDa. The buffer of the eluted proteins was changed to PBS and concentrated in a Ultrafree® centrifugal filter device (Millipore, Bedford, USA) with a cutoff size of 10 kDa.

Immunization of Lewis rats Lewis rats (8–10 weeks old) were obtained from the Laboratory Animal Science Division of the Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea. Immunization with the recombinant protein was performed by the following scheme: 1st immunization with 10 μg of recombinant protein emulsified in complete Freund's adjuvant (Sigma, St. Louis, USA); after 14 days, 2nd immunization with recombinant protein in incomplete Freund's adjuvant; after 10 days, 3rd immunization with 10 μg recombinant protein in incomplete Freund's adjuvant. Test bleeding for checking the antiserum titer was performed every 3 days after the 2nd and 3rd immunization. Animals showing high titer against the immunized protein were sacrificed by cervical dislocation. The coagulated blood was down-centrifuged and the supernatant containing the antiserum was retained.

ELISA The specificity and titer of the rat antiserum was determined by ELISA. The day before analysis, 96-well round bottom MaxiSorb™ ELISA plates (NUNC, Roskilde, Denmark) were each coated with 1 μg of the recombinant protein in coating buffer (0.1 M Na-carbonate, pH 9.5) per well. On the next day, the coating agents were removed by extensive washing with TBS/0.05% Tween-20, and nonspecific binding was blocked by incubation with 3% casein in TBS for 1 h at room temperature. After removal of the blocking agent, antiserum or pre-immune serum was added to the corresponding wells in a serially diluted manner. Specific binding of antibodies was detected after serum incubation for 1 h at room temperature using horseradish peroxidase conjugated anti-rat IgG antibodies (Sigma, St. Louis, USA) with subsequent addition of the corresponding substrates for colormetric analysis (*o*-phenylenediamine and H_2O_2).

Western blot analysis Whole cell lysates of each 5×10^6 Wa-cells were separated on a 12.5% SDS polyacrylamide gel under reducing and nonreducing conditions. After gel running, protein bands were transferred by electroblotting onto a Hybond™ ECL™ Nitrocellulose membrane (Amersham International plc, Buckinghamshire, England), followed by blocking of nonspecific binding with 3% casein. The membrane was then incubated with the newly generated rat antiserum overnight at 4°C. On the next day, excessive antibodies were washed out with TBS/0.05% Tween-20 and the bound primary antibodies detected with peroxidase conjugated anti-rat IgG antibodies (Sigma) and the ECL™ detection kit.

FACS analysis Specific binding of the rat immune serum to HLA-DRB1*0405-expressing cells was tested by FACS analysis using EBV-transformed B-cell lines with predefined HLA-haplotypes. For one staining reaction, 2×10^5 cells of each cell lines were incubated with the rat antiserum to a dilution of 1:1000 for 30 min at 4°C. Excessive reagents were washed out twice with PBS/azide (0.05%), and specifically bound antibodies were detected with biotinylated anti-rat Ig antibodies (Pharmingen, San Diego, USA) and phycoerythrin conjugated streptavidin (Pharmingen). Flow cytometry was performed on a FACScan™ (Becton Dickinson Inc., Mountain View, USA), and the obtained data were analyzed using the software program LYSYS™ (Becton Dickinson Inc., Lincoln Park, USA). Analysis of HLA-DRB1*0405 expression on peripheral blood lymphocytes was performed on peripheral blood lymphocytes (PBLs) isolated by a Ficoll-Histopaque®-1077 (Sigma) gradient from blood of healthy Korean individuals. Staining and FACS analysis were carried out in the same way as for those in the analysis of EBV-transformed B-cells, as described above.

Results and Discussion

Expression and purification of an allele-specific region of the HLA-DRB1*0405 The expression vector pQE-Ex2/B1*0405 encodes a 124 polypeptide with a 6 × histidine-tag onto its N-terminal end and contains an 87 a.a. fragment from the β1-region of the HLA-DRB1*0405 molecule (see Materials and Methods). As shown in Fig. 3, the recombinantly expressed protein was produced in the form of inclusion bodies. For the solubilization and rapid isolation, the induced recombinant proteins were purified after boiling of the isolated inclusion bodies by separation in a SDS-PAGE and subsequent electroelution in dialysis tubing. As determined by analytical gel electrophoresis (Fig. 3), the isolated protein was successfully purified as a single band without any contaminations. Since this recombinantly expressed allele-specific region is constructed as a fusion product with a polyhistidine tail, it is obvious that these products might also be alternatively purified by binding to chelating Ni^+-columns.

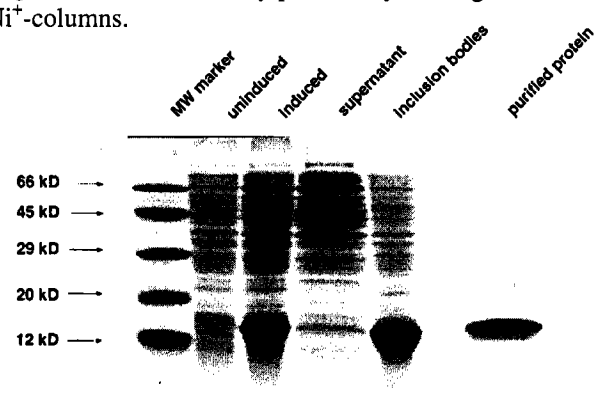

Fig. 3. SDS-polyacrylamide gel analysis of the expression and purification of the recombinant proteins. To the right of the molecular weight marker, whole lysate of IPTG induced and uninduced cells are shown in the lane labelled as "induced" and "uninduced". Under "inclusion bodies" and "supernatant" are shown the pellet and supernatant after sonication of IPTG induced cells. Purified proteins after gel elution are shown in the last lane.

Development of rat immune serum The recombinantly expressed region within the DRB1*0405 covered the most allele-specific region. Selective expression of this region was intended to minimize the possibility of raising antibodies to any conserved part of the DRβ chain, which would be the case in immunization of a more extended region or even the whole β-chain. Based on this fact, the region to be expressed was selected to cover the exon-2 region, which is the most variable part in DR alleles, as well as to contain certain amino acid residues which had been reported to be involved in determination of allele specific peptide binding motifs. For example, it had been observed that the amino acid residues which distinguish DR4 subtypes, and which are shared by RA-associated DR4 molecules, are located in the peptide binding site (McNicholl *et al.*, 1995), predominantly in the α-helical region of the DRβ chain (positions 37, 57, 67, 70, 71, 74, and 86). This was more drastically observed when comparing the sequence of DRB1*0405 to that of DRB1*0101, the major allele of the DR1 haplotype (Maeng *et al.*, 1997). But while the selective expression of this small epitope has the advantage to generate a targeted immune response toward this allele-specific region, since this region comprises only a small part (87 a.a.) of the whole β-chain, immunogenicity of this small polypeptide would be expected to be significantly lowered than the whole protein. To overcome this problem without extension of the DR β-chain derived sequences, the 87 a.a. region was expressed by flanking with vector derived sequences. As shown in Fig. 2 and described in Materials and Methods, using this 2-step cloning strategy, the originally 87 a.a. long polypeptide could be enlarged and also be provided with a poly-His tag, which ensured a convenient purification strategy as well as facilitated the detection of this recombinant protein by use of poly-His specific monoclonal antibodies which are commercially available.

Rats (strain Lewis) were immunized with the recombinant protein since they have the advantage over rabbits and mice in being easy to handle and are rich in providing immune sera. The specificity and activity of the antiserum were determined by an enzyme-linked immunosorbent assay. As shown in Fig. 4, in contrast to normal rat serum, the newly generated antiserum revealed a high and saturable binding activity to recombinant proteins coated on 96-well plates. To confirm the specific binding of the newly generated rat antibodies to MHC class II molecules, especially to the β-chain, Western blot analysis was performed with this antiserum. Wa-cells expressed high levels of class II molecules, and it was expected to produce a specific signal band around 28 kD which is the expected molecular weight of the class II β-chain. As shown in Fig. 5, under the reducing condition, a single band was indeed detectable by the antiserum. Since the MHC class II complex forms an ~60 kDa heterodimeric complex, it was also expected to detect the dimeric complex, but only a trace amount of signal was to be observed, indicating either the relatively unstable state of the complex under these blotting and detection conditions, or the disability of the immune serum to detect its epitope under nonreducing conformations.

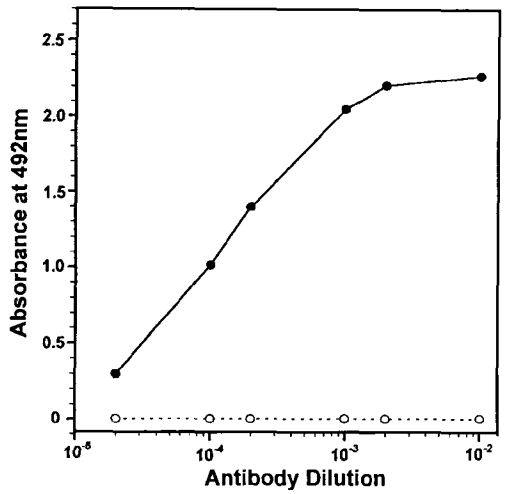

Fig. 4. Determination of antigen specific binding of the rat antiserum by ELISA. ELISA plates were coated with 1 μg of the immunized peptide and incubated with either the immune serum or normal rat serum in a serially diluted manner. Closed and open circles indicate binding of the rat antiserum and normal rat serum, respectively.

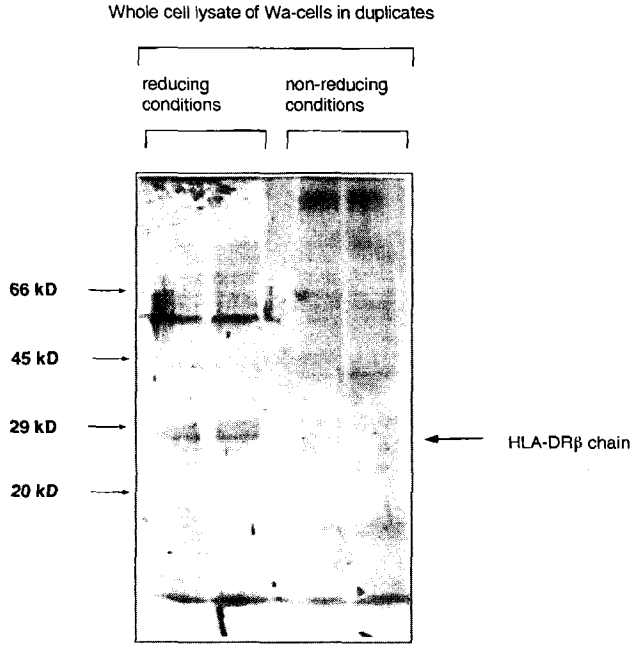

Fig. 5. Western blot analysis of MHC class II expression on Wa-cells detected by the rat antiserum. Whole cell lysates of each 5×10^6 Wa-cells were separated in duplicate either under reducing (left) or nonreducing (right) conditions. The blot was developed with the rat antiserum and the ECL^{TM} system.

FACS analysis of EBV-transformed B-cell lines with predefined HLA haplotypes The rat antiserum generated in this study showed high reactivity in ELISA and Western blot against the denatured form of the β1-domain of the HLA-DRβ-chain. But, in the case where the antigen specificity of these antibodies are restricted only to a linear epitope, the application of this antiserum would be largely limited to the detection of denatured antigens. Therefore, to determine their binding capacity to correctly folded HLA-DR4 molecules on the cell surface, Wa-cells were stained with this antiserum and the specifically bound antibodies detected by fluorescence labelled secondary reagents. FACS analysis revealed that MHC-class II molecules were indeed identified by this immune serum (Fig. 6A), whereas, on the contrary, the fibroblast HeLa cell line, which are devoid of class II molecules, were not stained. To confirm the specificity of the newly generated antiserum, another cell line with a previously-determined HLA haplotype was analyzed. The B-cell line, KT, was established by EBV-transformation of peripheral blood lymphocytes and was kindly provided by Dr. H. Han (Catholic Medical School, Seoul, Korea). By PCR-SSP, this cell line was determined to express HLA-DR molecules of the HLA-DRB1*12, HLA-DRB1*13 haplotypes.

By FACS analysis of this cell line, as shown in Fig. 6A, qualitative differences were observed in the staining patterns between the two cell lines, KT and Wa. The slightly positive signal in KT cell lines, despite missing HLA-DR4 molecules, is thought to result from the binding of some antibodies in the immune serum which were generated by immune reactions against conserved HLA-DR motifs. Nevertheless, the higher signal intensity on the DRB1*0405 homozygotic Wa-cells indicated the preferential staining of DR4-specific motifs of these antibodies. Solid-phase absorption of cross-reacting antibodies on DR molecules of other haplotypes will generate a more specific antiserum, from which the resolution of allelic specificity might then be further determined. The degree of resolution whereby this antiserum can determine allelic variations will need further investigations with other cell lines expressing similar but nonidentical DR sequences to that of the immunized DRB1*0405.

Detection of HLA-DR4 expression on PBLs Analysis of HLA-DR molecule expression by cell surface staining has the advantage over that by RT-PCR in its faster processing time and convenient handling. It is also a more economical method in terms of the dependency on expensive reagents such as reverse-transcriptase and thermostable polymerase. Furthermore, antibody staining and analysis by fluorescence activated flow cytometry have the advantage to determine simultaneously the expression level of MHC class II molecules as well as,

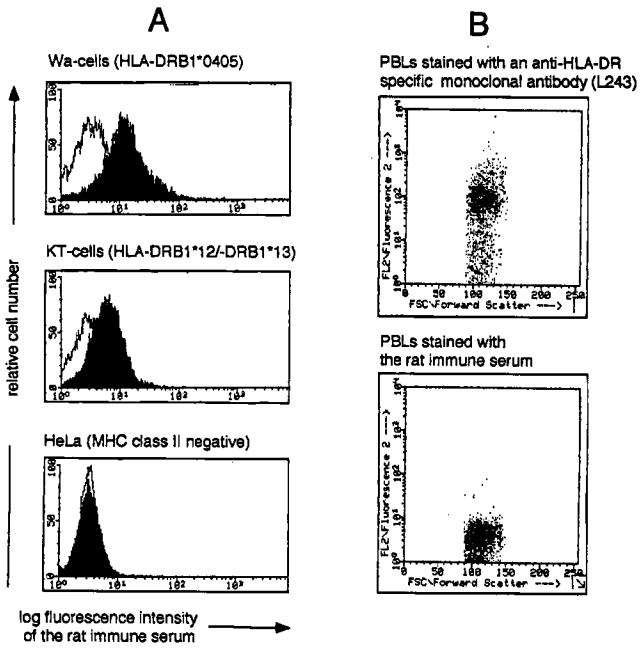

Fig. 6. FACS analysis of EBV-transformed B-cells of various HLA alleles and PBLs with the newly generated antiserum. Each 2×10^5 cells were stained for analysis. A. The histograms in the left panel show the results from FACS analysis of EBV-transformed cell lines with different DR haplotypes, and as a control that of the fibroblast cell HeLa which do not express class II molecules at all. Filled histograms indicate cells stained with the rat antiserum, and open histograms show the fluorescence distribution of cells stained with a control rat serum. B. The dot-plot diagrams show the FACS analysis data from PBLs stained either with an anti pan-HLA-DR monoclonal antibody (L243) or with the rat antiserum. The horizontal axis indicates the cell size, and the vertical axis shows the fluorescence signal intensity of either the L243 monoclonal antibody or that of the newly generated rat antiserum.

when combined with other antibodies, to examine several parameters of cell surface marker expression at once.

To show that not only cell lines but also primary cells can be determined on their MHC class II expression by this antiserum, peripheral blood lymphocytes (PBLs) were isolated, stained, and analyzed following the same scheme as for EBV-transformed B-cells. A representative result is shown in Fig. 6B. In these dot-plot diagrams, FACS analyses data are shown from PBLs stained with either the anti pan-HLA-DR monoclonal antibody, L243, or with the newly generated rat antiserum. As shown in the L243 dot-plot, a large subpopulation comprising the B-lymphocytes and monocytes were stained positively with this antibody indicating the high expression level of MHC class II molecules on these cells. Nevertheless, no staining was observed on the same PBLs incubated with the rat antiserum. Regarding the fact that this antiserum can indeed recognize allele-specific motifs on MHC class II molecules (Figs. 4 and 5), it is obvious that, in the case of

this specific donor, no HLA-DR4 are expressed on the cell surface of MHC class II positive cells. These observations confirm the heterogeneous expression of HLA-DR molecules among different individuals, and indicates the capacity of this antiserum to discriminate allelic variations on the cell surface level. It is evident that these novel antibodies will become a convenient and powerful tool when applied in analyses of HLA-DR molecules, and especially those of the DR4, upon their induction, regulation, and cell surface expression in connection with several genetic factors, as well as in autoimmune and infectious diseases. In summary, the recombinant protein and the antibodies generated in this study will serve as potent utilities in studying DR4 molecules as well as in development of further monoclonal antibodies.

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References

Alper, C. A., Kruskall, M. S., Bagley-Marcus, D., Craven, D. E., Katz, A. J., Brink, S. J., Dienstag, J. L., Awdeh, Z., and Yunis, E. J. (1989) Genetic prediction of nonresponse to hepatitis B vaccine. *New Engl. J. Med.* **321**, 708–712.

Blackman, M. A., Marrack, P., and Kappler, J. (1989) Influence of the major histocompatibility complex on positive thymic selection of V beta 17a+ T cells. *Science* **244**, 214–217.

Choi, Y., Marrack, P., and Kappler, J. W. (1992) Structural analysis of a mouse mammary tumor virus superantigen. *J. Exp. Med.* **175**, 847–852.

Cresswell, P. (1994) Assembly, transport, and function of MHC class II molecules. *Annu. Rev. Immunol.* **12**, 259–293.

Gaudieri, S., Leelayuwat, C., Tay, G. K., Townend, D. C., and Dawkins, R. L. (1997) The major histocompatability complex (MHC) contains conserved polymorphic genomic sequences that are shuffled by recombination to form ethnic-specific haplotypes. *J. Mol. Evol.* **45**, 17–23.

Gorski, J. (1989) HLA-DR beta-chain polymorphism. Second domain polymorphism reflects evolutionary relatedness of alleles and may explain public serologic epitopes. *J. Immunol.* **143**, 329–333.

Imanishi, T., Akaza, T., Kimura, A., Tokunaga, K., and Gojobori, T. (1992) Allele and haplotype frequencies for HLA and complement loci in various ethnic groups, in *HLA 1991 Vol. 1.* Tsuji, K., Aizawa, M., and Sasazuki, T. (eds.), pp. 397–419, Oxford University Press, Oxford.

Kang, J. H., Maeng, C. Y., Park, J. -H., Hahm, K. S., Han, B. D., and Kim, K. L. (1997) Reassembly and reconstitution of separate α and β chains of human leukocyte antigen DR4 molecule isolated from *Escherichia coli. Mol. Cells* **7**, 237–243.

Kappes, D. and Strominger, J. L. (1988) Human class II major histocompatibility complex genes and proteins. *Annu. Rev. Biochem.* **57**, 991–1028.

Kim, H. Y., Min, J. K., Yang, H. I., Park, S. H., Hong, Y. S., Jee, W. H., Lee, S. H., Cho, C. S., Kim, T. G., and Han, H. (1997) The impact of HLA-DRB1*0405 on disease severity in Korean patients with seropositive rheumatoid arthritis. *Br. J. Rheumatol.* **36**, 440–443.

Maeng, C. Y., Kang, J. H., Park, J.-H., Hahm, K. S., and Kim, K. L. (1997) Cloning and nucleotide sequence analysis of HLA-DRA*0101 and DRB1*0405 alleles. *Korean J. Immunol.* **19**, 17–27.

Matsushita, S., Takahashi, K., Motoki, M., Komoriya, K., Ikagawa, S., and Nishimura, Y. (1994) Allele specificity of structural requirement for peptides bound to HLA-DRB1*0405 and -DRB1*0406 complexes: implication for the HLA-associated susceptibility to methimazole-induced insulin autoimmune syndrome. *J. Exp. Med.* **180**, 873–883.

McNicholl, J. M., Whitworth, W. C., Oftung, F., Fu, X., Shinnick, T., Jensen, P. E., Simon, M., Wohlhueter, R. M., and Karr, R. W. (1995) Structural requirements of peptide and MHC for DR (α, β 1*0401)-restricted T cell antigen recognition. *J. Immunol.* **155**, 1951–1963.

Mollick, J. A., Cook, R. G., and Rich, R. R. (1989) Class II MHC molecules are specific receptors for *Staphylococcus* enterotoxin A. *Science* **244**, 817–820.

Moribe, T., Kaneshige, T., Hirakata, M., Mimori, T., Akitsuki, M., and Inoko, H. (1996) Identification of a DRB1*0405 variant (DRB1*04052) using the PCR-RFLP method. *Tissue antigens* **47**, 450–453.

Pierce, S. K., Morris, J. F., Grusby, M. J., Kaumaya, P., van Buskirk, A., Srinivasan, M., Crump, B., and Smolenski, L. A. (1988) Antigen-presenting function of B lymphocytes. *Immunol. Rev.* **106**, 149–180.

Savage, D. A., Tang, J. P., Wood, N. A. P., Evans, J., Bidwell, J. L., Wee, J. L. K., Oei, A. A., and Hui, K. M. (1996) A rapid HLA-DRB1*04 subtyping method using PCR and DNA heteroduplex generators. *Tissue Antigens* **47**, 284–292.

Shindo, Y., Ohno, S., Yamamoto, T., Nakamura, S., and Inoko, H. (1994) Complete association of the HLA-DRB1*04 and -DQB1*04 alleles with Vogt-Koyanagi-Harada's disease. *Hum. Immunol.* **39**, 169–176.

Steinman, R. M. (1991) The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol.* **9**, 271–296.

Stern, L. J., Brown, J. H., Jardetzky, T. S., Gorga, J. C., Urban, R. G., Strominger, J. L., and Wiley, D. C. (1994) Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. *Nature* **368**, 215–221.

Svensson, A. C., Setterblad, N., Pihlgren, U., Rask, L., and Andersson, G. (1996) Evolutionary relationship between human major histocompatibility complex HLA-DR haplotypes. *Immunogenetics* **43**, 304–314.

Swain, S. L., Dialynas, D. P., Fitch, F. W., and English, M. (1984) Monoclonal antibody to L3T4 blocks the function of T cells specific for class II major histocompatibility complex antigens. *J. Immunol.* **132**, 1118–1123.

Unanue, E. R. (1984) Antigen-presenting function of the macrophage. *Annu. Rev. Immunol.* **2**, 395–428.

Varla-Leftherioti, M., Papanicolaou, M., Spyropoulou, M., Vallindra, H., Tsiroyianni, P., Tassopoulos, N., Kapasouri, H., and Stavropoulos-Giokas, C. (1990) HLA-associated non-responsiveness to hepatitis B vaccine. *Tissue Antigens* **35**, 60–63.

Winslow, G. M., Marrack, P., and Kappler, J. W. (1994) Processing and major histocompatibility complex binding of the MTV7 superantigen. *Immunity* **1**, 23–33.

Wordsworth, B. P. and Bell, J. I. (1992) The immunogenetics of rheumatoid arthritis. *Springer Semin. Immunopathol.* **14**, 59–78.