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Polymorphism and complexity of the human DC and murine I-A α chain genes

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A cDNA clone encoding the human B cell alloantigen DC α chain (pDCH1) has been used to analyse the structure of the human and murine major histocompatibility complexes by the DNA filter hybridization technique. The pDCH1 probe hybridizes to a single DNA sequence present on chromosome 17 in the mouse genome. A restriction enzyme polymorphism enables us to map this sequence to the I-A subregion. Extensive restriction enzyme polymorphism detected in HLA-DR homozygous typing cells is reminiscent of the DR/MT linkage disequilibrium groups, suggesting that the pDCH1 probe could be useful for haplotype typing in the human population. The HLA-DR region appears more complex than the I region since a second DC-like hybridizing sequence is detected in the human genome in these experiments.

Key words: class II antigens/DNA filter hybridization/major histocompatibility complex/restriction enzyme polymorphism

Introduction

The major histocompatibility complex (MHC) is located on chromosome 17 in mouse and on chromosome 6 in man. It encodes a variety of proteins involved in immunological processes (Klein, 1979). Among them, the class II antigens which are primarily expressed on B lymphocytes, are encoded in the I region in mouse and in the DR region in man. They consist of two non-covalently associated polypeptide chains named the α (heavy) and β (light) chains which both pierce the membrane (reviewed by Murphy, 1981; Strominger *et al.*, 1981). The class II antigens are thought to be the product of the Ir genes and mediate self-restricted cell-cell interactions and antigen presentation (Benacceraf, 1981).

The availability of recombinant strains of mice has allowed a division of the I region into several subregions. Two of these subregions are the I-A subregion containing the Aα, Aβ and Eβ (Ae) chain genes, and the I-E subregion containing the Eα chain gene (Cook *et al.*, 1979). The human HLA-DR antigen has been shown to correspond to the murine I-E antigen. In both species, the β chains are highly polymorphic, whereas the Eα and HLA-DRα chains are invariant chains (reviewed in Shackelford *et al.*, 1982). Among the other class II antigens that have been identified, the DC1 antigen (Tosi *et al.*, 1978), which is found in strong linkage disequilibrium with DR1,2 and w6 (Park *et al.*, 1980), is identical to MB1 and MT1 and constitutes a class II antigen molecule distinct from HLA-DR (Shackelford *et al.*, 1981). Recently, the amino-terminal sequence of DC α chains has been obtained (Bono and Strominger, 1982; Goyert *et al.*, 1982) suggesting a close relationship with the murine I-Aα chain. In the I-A and DC antigens, both the α and β chains are polymorphic, although the polymorphism of the α chains is limited (Cook *et al.*, 1979; De Kretser *et al.*, 1982). The linkage between various human diseases, mainly autoimmune diseases, and particular HLA-DR specificities has been widely documented (Svejgaard *et al.*, 1980). However, it appears that in certain cases, the disease susceptibility is more closely related to class II molecules serologically or functionally defined as distinct from HLA-DR molecules (Reinertsen *et al.*, 1978; Moutsopoulos *et al.*, 1979). Therefore, accurate typing for these antigens might prove useful in clinical studies.

New insights into the molecular organization of the I and HLA-DR regions have been made possible recently by the isolation of cDNA clones encoding the HLA-DRα chain (Lee *et al.*, 1982; Korman *et al.*, 1982; Larhammar *et al.*, 1982) and a DR-like β chain (Wiman *et al.*, 1982; Long *et al.*, 1982) as well as the I-Aβ chain (R.N.Germain and J.G.Seidman, unpublished data). We have reported the isolation and nucleotide sequence of a cDNA clone corresponding to the DCα chain (pDCH1) (Auffray *et al.*, 1982). Here we report the use of pDCH1 to probe the murine and human genomes by the filter hybridization technique. The results presented show that the mouse counterpart of the human DCα chain gene maps to the I-A subregion on chromosome 17. Analysis of cells typed as HLA-DR homozygous provides evidence that two DC-like α chain sequences are present in the human genome. The DCα chain gene shows a restriction enzyme polymorphism that could prove useful in haplotype typing in human populations.

Results

The DCα chain gene is analogous to a murine gene that is located in the I-A subregion of chromosome 17

The precise relationship between the murine Ia antigen genes and the DCα chain gene has not yet been determined. The structures of the murine Ia antigen chains and their genes are not completely defined and therefore complete structural comparison of the human DCα chain to the murine Ia antigens cannot be made. The DCα chain gene is homologous to a single murine gene because the former hybridizes to a single restriction fragment in mouse DNA (Figure 1, lane 1). The existence of a somatic cell hybrid and congenic recombinant inbred mouse strains permits the mapping of the mouse analogue of the DCα chain gene to a specific mouse chromosome and to a genetic region on this chromosome.

Since we suspected that the murine analogue of the DCα chain gene would be encoded in the murine MHC, we hybridized the DCα chain probe to the DNA of a somatic cell hybrid (Smiley *et al.*, 1978) containing only murine chromosome 17 and a full complement of Chinese hamster chromosomes (Figure 1). The restriction fragment found in C3H mouse DNA is found in the somatic cell hybrid, but not in Chinese hamster DNA.

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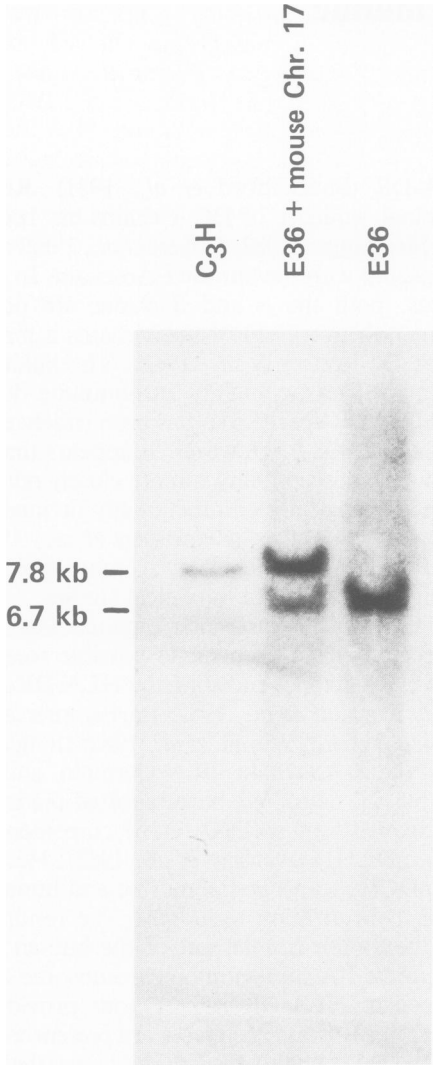

Fig. 1. Analysis of a somatic cell hybrid containing the murine chromosome 17. DNA from mouse (C3H), a chromosome 17-containing mouse/Chinese hamster cell line (E36 + Chr17) and the hamster parent line (E36) was digested with *Bam*HI, fractionated on a 0.9% agarose gel, transferred to a nitrocellulose filter and hybridized to the pDCH1 insert as described in Materials and methods. The size of the hamster and mouse derived fragments were determined by comparison to λ *Hind*III restriction fragments (not shown).

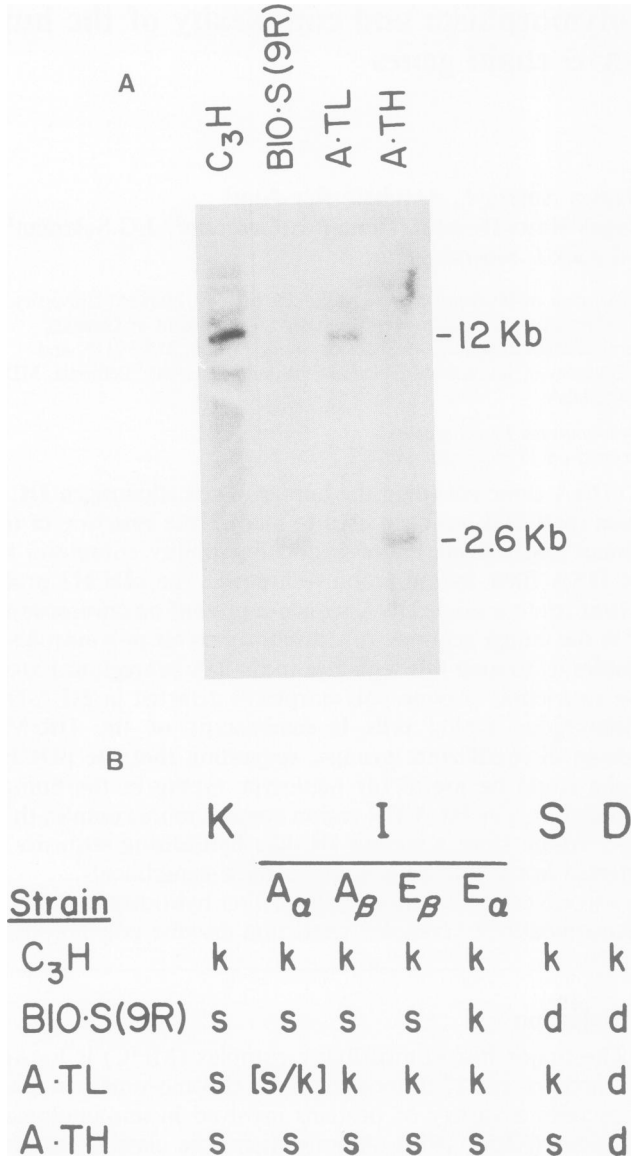

Fig. 2. Analysis of mouse congenic recombinant inbred strains. **(A)** DNA from the different mouse strains was digested with *Hind*III, and analysed as indicated in legend of Figure 1. The sizes of the H-2^s derived (2.6 kb) and H-2^k derived (12 kb) fragments were determined by comparison to λ *Eco*RI restriction fragments (not shown). **(B)** The haplotypes, defined by serological and functional analysis of the four mouse strains, are indicated (Klein, 1975).

The mouse analogue of the DCα chain gene can also be mapped to a specific region of chromosome 17, thanks to its location on a polymorphic restriction fragment. Although *Bam*HI restriction fragments were not polymorphic, *Hind*III fragments were polymorphic in three H-2^s, H-2^k and H-2^b haplotypes (data not shown). The gene is encoded on a 12-kb restriction fragment in C3H (I-A^k) mice and 2.6-kb restriction fragment in A.TH (I-A^s) mice (Figure 2, panel A). Since congenic recombinant inbred mice that have undergone recombination within the MHC exist, these polymorphic restriction fragments can be mapped (Figure 2, panel B) to a subregion of the MHC. For example, the mouse analogue of the DCα chain gene maps to the left of the I-E subregion because the 2.6-kb fragment is found in B10.S(9R) DNA as well as in A.TH DNA. Similarly, it maps to the right of the K region because the 12-kb fragment is found in A.TL DNA as well as in C3H DNA. Thus, the data presented in Figure 2 show that the hybridizing restriction fragment in the H-2^s haplotype maps to the I-A subregion.

Restriction enzyme polymorphism of the DCα chain gene

The DNA from six HLA-DR homozygous cell lines was analysed by the filter hybridization technique with the pDCH1 probe (Figure 3). When the filter was washed under conditions which do not allow cross-hybridization with the DRα chain gene, two DNA fragments were detected with all six cell lines. The small fragment is of the same size (2.6 kb) in five of them. The large fragment shows a more complex pattern but is of the same size in certain cell lines. The possibility that the two fragments represent different parts of a single gene is unlikely since the 2.6-kb *Hind*III fragment (provisionally called DX) and the 5.5-kb *Hind*III fragment detected in the DR4,4 cell line are present in different non-

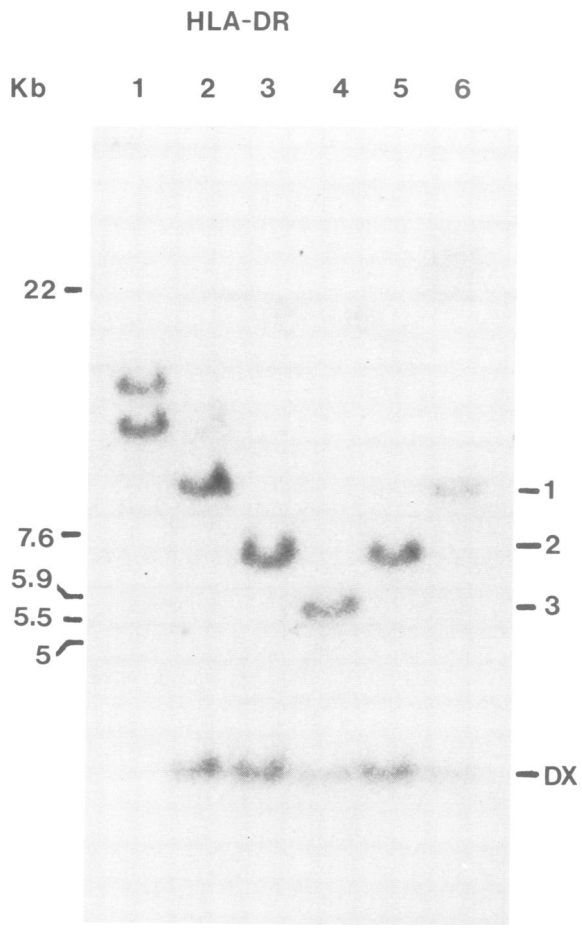

Fig. 3. Analysis of HLA-DR homozygous typing cells. DNA from six lymphoblastoid B cell lines homozygous for HLA-DR (see Materials and methods) was digested with *Hind*III and analysed as described in Materials and methods with the pDCH1 probe. The number of each lane corresponds to the DR specificity. The size in kilobases (kb) of λ *Eco*RI restriction fragments is indicated on the left. The numbers on the right refer to the three groups defined in the text.

overlapping genomic clones. Moreover, it is excluded by sequence data (unpublished data) and we have shown in a separate study that the large fragment corresponds to the DCα chain gene, which was localized on chromosome 6 by using DR-null and DC/MT-null deletion loss mutants. The parental DR1 haplotype from which these mutants are derived, shows a pattern of *Hind*III fragments corresponding to that observed in the DR2,2 and DRw6,w6 cell lines studied here (Auffray *et al.*, in preparation). Therefore, with the exception of DR1 in IBW4, the DR haplotypes can be classified into three groups according to the size of the large *Hind*III fragment: (1) DR1,2 and w6: 9.5 kb; (2) DR3 and 5: 7 kb and (3) DR4: 5.5 kb (Figure 3). These groups are strikingly reminiscent of the DR/MT linkage disequilibrium groups in which MT1 is linked to DR1,2 and w6; MT2 is linked to DR3, 5,w6 and w8, and MT3 is linked to DR4, 7 and w9 (Park *et al.*, 1980).

Discussion

Mouse recombinant strains have facilitated dissecting the H-2 complex into regions and subregions. Biochemical analysis of the products of the H-2 and HLA complexes has indicated the high sequence homology existing between the polypeptide chains encoded by the corresponding loci (Orr *et al.*, 1979; Tragardh *et al.*, 1980; Coligan *et al.*, 1981). These findings have been extended with the isolation of molecular probes for the corresponding genes. In fact, extensive nucleotide sequence homology has allowed use of human probes to isolate mouse genes (Steinmetz *et al.*, 1981; Evans *et al.*, 1982) and *vice versa* (Jordan *et al.*, 1981). Using the potential cross-hybridization of human and mouse class II gene sequences, it might be possible to correlate a new human DNA probe such as pDCH1 with its mouse counterpart and define subregions in the HLA-DR region. Here, we have shown that the cDNA clone pDCH1, which encodes the DCα chain (Auffray *et al.*, 1982), hybridizes to a DNA fragment present on the mouse chromosome 17 by using a mouse/Chinese hamster somatic cell hybrid containing only this particular chromosome. Furthermore, this experiment shows that a single fragment is detected in both the mouse and Chinese hamster genome thanks to extensive cross-hybridization, suggesting that the corresponding genes have been highly conserved throughout evolution. The murine analogue of the DCα chain gene can be mapped to the I-A subregion because it is encoded on a polymorphic restriction fragment. The precise location of the murine gene within the I-A subregion cannot be determined by this approach since the appropriate mouse strains do not exist. That is, one might be able to map the murine DCα-like gene to a region closer to a particular I-A chain gene if strains existed which had undergone recombination between these genes. Although two intragenic I-A recombinants exist (A.TL and B10.GD), they do not allow the exclusion of any of the I-A subregion genes. Nevertheless, the results presented in Figures 1 and 2 establish that there is a single murine gene in the I-A subregion of chromosome 17 which is closely related to the DCα chain gene. We have also mapped the murine analogue of the DCα chain gene to the I-A subregion in the H-2^b haplotype (not shown). Because only one I-Aα chain is known to be encoded in this region, we suggest that the I-Aα chain gene is the murine analogue of the human DCα chain gene. This observation agrees with previous evidence provided by comparison of amino-terminal amino acid sequences of the DC and I-Aα chains (Bono and Strominger, 1982; Goyert *et al.*, 1982). The isolation and characterization of the I-Aα chain gene will allow us to confirm this relationship.

Using the same probe, we have analysed six cell lines typed as HLA-DR homozygous, five of which are also homozygous at the HLA-A,B loci (see Materials and methods), but have not been serologically typed for class II antigens other than HLA-DR. In the case of the DR1,2,3 and w6 cell lines which have been derived from offspring of consanguinous marriages, it can be suspected that they are also homozygous at the closely linked locus studied here.

The restriction enzyme polymorphism of the DCα chain gene can be interpreted only with caution because of the small number of cell lines studied. However, since the pattern of bands is very simple and is reminiscent of the DR/MT linkage disequilibrium groups, the data can be tentatively explained by one of the following hypotheses: (1) the MT1,2 and 3 determinants are, at least in part, located on the DCα chain and the restriction enzyme polymorphism of the DCα chain gene is a direct reflection of allelic differences; (2) the DCα chain gene is closely linked to one or several β chain genes encoding the MT determinants and has a related polymorphism. In the first case, it is expected that the allospecific differences will be located inside the coding sequence in the various DNA fragments detected here. The availability of

DC-like β chain cDNA clones (Wiman *et al.*, 1982; Long *et al.*, 1982) will allow us to address the second possibility.

The difficulty of using alloantisera for locus-specific typing is emphasized by the fact that the MT2 determinant is not only present on class II molecules distinct from HLA-DR but also on DR5 molecules (Karr *et al.*, 1982). Even when monoclonal antibodies are used, an epitope found on a DC-like molecule in one homozygous cell line can be found on HLA-DR molecules in other homozygous cell lines (Goyert *et al.*, 1982). With such reservations, the MT1,2 and 3 specificities appear to constitute an allelic series. It is worth noting that the DR1,1 homozygous cell line IBW4, which shows a DCα gene restriction fragment of a different size than expected for group 1, is also the only one in which the DX fragment is of a different size. This may simply reflect the fact that the DX fragment is closely linked to the DCα chain gene. If so, the human DC subregion will appear to be more complex than the mouse I-A subregion.

In our view, a more reliable typing for the class II antigens will be achieved when the correspondence between a DNA fragment, a functional gene encoding a class II molecule α or β chain and an antigenic determinant defined by specific alloantisera, monoclonal antibodies or alloreactive primed lymphocyte typing (PLT) clones is established. Before such complete information is available, we hope that by extending the present study to a large panel of individuals, the pDCH1 probe will prove to be a useful tool for typing individuals for the class II antigen(s) encoded in the DC subregion.

Materials and methods

Human cell lines

The lymphoblastoid B cell lines were obtained from the Genetics Laboratory, Oxford University and maintained as long-term cultures in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin (all obtained from Grand Island Biological Co., Grand Island, NY). These cell lines were typed for HLA-A,B,C and DR as follows: (1) IBW4: A1,1; B35,35; Cw4,w4; DR1,1 (2) PGF: A3,3: B7,7; DR2,2 (3) WT49: A2,2; B15,15; Cw3,w3; DR3,3 (4) Priess: A2,2; B15,15; Cw3,w3; DR4,4 (5) Mich: A2,32; B7,15; DR5,5 (6) WT46: A32,32; B13,13; DRw6,w6.

Mouse and mouse/Chinese hamster lines

The cell lines R44.1 (E36 Chinese hamster and mouse chromosome 17) and E36 (Chinese hamster) were those described by Smiley *et al.* (1978) and were generously provided by Peter d'Eustachio. The recombinant inbred lines B10S(9R), A.TH and A.TL were kindly provided by David Sachs and Jay Bersofsky. C3H mice were obtained from Jackson Laboratories.

DNA filter hybridization analysis

High mol. wt. DNA was extracted from the six cells typed as HLA-DR homozygous essentially as described (Blin and Stafford, 1976). After complete digestion with *Hind*III (New England Biolabs), the DNA fragments were separated by electrophoresis on a 0.5% agarose gel and transferred to a nitrocellulose filter (Schleicher and Schuell) as described (Southern, 1975). The pDCH1 insert (Auffray *et al.*, 1982) was labeled by nick-translation (Rigby *et al.*, 1977), at a specific activity of 10^8 c.p.m./μg and used to probe the filter as described (Heidmann and Rougeon, 1982) with the addition of 10% dextran sulfate (Pharmacia). The filter was washed as follows: 1 h at 65°C in 2 x SET (1 x SET = 0.15 M NaCl, 1 mM EDTA, 50 mM Tris, pH 7.8), and 2 h at 65°C in 1 x SET, then dried and exposed to an XAR-5 Kodak X-ray film with an intensifying screen for 2 days. The analysis of mouse and mouse/Chinese hamster DNA was performed under conditions previously described (Margulies *et al.*, 1982) using the same probe as above. The filters were washed in non-stringent conditions: the final wash was in 0.03 M Na citrate, 0.03 M NaCl (0.2 x SSC) at 42°C.

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