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A physical map linking the five CD1 human thymocyte differentiation antigen genes

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Human CD1 is a family of thymocyte differentiation antigens which consist of heavy chains with mol. wts between 43 and 49 kd binding to β2 microglobulin. They are distant relatives of the major histocompatibility complex (MHC) class I and II products. Five human CD1 genes have been described. Three (*CD1A*, *-B* and *-C*) code for the serologically defined CD1a, -b and -c antigens. The protein products of the other two genes, *CD1D* and *CD1E*, remain unknown. All CD1 genes are located on chromosome 1 and hence are independent of the MHC locus. In this paper, the tight linkage of the CD1 genes has been established by pulse field gel electrophoresis, cosmid cloning and walking techniques. The 190 kb of DNA linking all five CD1 genes has been spanned by 14 overlapping cosmids. The order of the genes in the CD1 complex is *CD1D-CD1A-CD1C-CD1B-CD1E*, and, with the exception of *CD1B*, they are arranged in the same transcriptional orientation. The genes are evenly spaced in the complex except for the distance between *CD1D* and *CD1A*, which is two to three times greater than the average.

Key words: cosmid cloning and walking/human genome/MHC/pulse field gel electrophoresis

Introduction

Human CD1 is a group of thymocyte differentiation antigens which are similar to the major histocompatibility complex (MHC) class I and class II products both in protein primary structure and in genomic exon-intron organization. Five different CD1 genes have been detected and cloned; and of these, three have been identified as encoding the CD1a, -b and -c antigens. The other two genes, described previously as *R2* and *R3* and referred to as *CD1E* and *CD1D* respectively here, may also code for functional products. This is because the mRNA for these two genes have been detected (Milstein *et al.*, 1987) and no nonsense mutations have been found in their cDNA and/or genomic DNA sequences (Balk *et al.*, 1989; Calabi *et al.*, 1989a). There is a variation in the number of CD1 genes present in different mammalian genomes. Rodents have only two, but rabbits may well contain eight or more CD1 genes (Bradbury *et al.*, 1988; Calabi *et al.*, 1989a). The human *R3* (*CD1D*) gene is more homologous to the mouse CD1 than to the other four (classic) human CD1 genes in the leader, α1 and α2 exons, and further sequence analysis with two rabbit CD1 genes has allowed categorization of CD1 genes into two classes, separating the *R3* (*CD1D*) from the *CD1A*, *-B*, *-C* classic genes. Both classes are found in the human and rabbit genomes, but the two murine genes are both of the *R3* (*CD1D*) class (Calabi *et al.*, 1989a,b). The functional role of CD1 has yet to be discovered. However, the evolutionary conservation of the genes suggests an essential role; the tissue-specific expression of CD1a, -b and -c in cortical thymocytes, CD1a in the Langerhans cells of the skin (Fithian *et al.*, 1981a) and CD1c in a fraction of B cells (Small *et al.*, 1987; Delia *et al.*, 1988) suggests an association with the immune system. Further indication for such a role derives from the involvement of β2 microglobulin with CD1a (Ziegler and Milstein, 1979), -b and -c, and CD8 (Ledbetter *et al.*, 1985; Small *et al.*, 1987) or HLA class I molecules with CD1a (Amiot *et al.*, 1988).

Neither human nor mouse CD1 genes are located in the MHC locus (Calabi and Milstein, 1986; Bradbury *et al.*, 1988). The human genes have been mapped to chromosome 1q22-23 (Albertson *et al.*, 1988). It is of particular interest to determine whether the CD1 genes cluster in a tight group or in two or more groups, as those for the MHC class I and II genes. Here we establish the linkage of the five human CD1 genes by pulse field gel electrophoresis (PFGE), cosmid cloning and walking techniques. A contiguous stretch of DNA spanning ~190 kb that contains the five human CD1 genes has been cloned and analysed.

In this paper the human five CD1 genes are designated as *CD1A-E*, according to the suggestion by the Nomenclature Committee for Human Gene Symbols. The known protein products are referred to as CD1a, -b and -c, following the international convention for the CD antigens. We adopted the designation of *CD1D* and *CD1E* for the formerly named *R3* and *R2* genes, whose protein products have not been described. This may require a revision because *CD1D* and, to a lesser extent, *CD1E* are evolutionarily quite distinct from *CD1A*, *-B* and *-C* (Calabi *et al.*, 1989b; see below). Until more is known about the cellular distribution and function of the putative protein products for *CD1D* and *CD1E*, their designation as CD1 antigens remains premature.

Results

Linkage of CD1 genes by PFGE

Initial evidence for linkage of the human CD1 genes was obtained by the PFGE technique. Specific DNA probes for various CD1 genes were derived from the 3′ untranslated regions from cloned cDNA (*CD1A*, *-B* and *-D*) or genomic DNA (*CD1C* and *-E*). As shown in Figure 1, *CD1A-D* probes (the *CD1E* probe was not used at this stage) hybridized to identical 240-260 kb *Xho*I (lanes 4 and 8), and 700-760 kb *Sfi*I (lanes 3 and 7) restriction fragments. These implied that at least these four CD1 genes cluster in a small region <260 kb in size. *Sal*I digests divided the five genes into two groups. *CD1A* and *CD1D* probes hybridized to a 550 and 600 kb doublet (Figure 1, lane 1) and an ~180 kb

(Figure 2, lane 1; with an apparent size of 250−300 kb in Figure 1, lane 1) *Sal* fragments, while the CD1B−C and -*E* probes hybridized only to an ~600 kb *Sal*I fragment (Figure 1, lane 5). Further Southern blot analysis of *Sal*I−*Xho*I double-digested DNA is shown in Figure 2. The *CD1C* probe hybridized to an ~80 kb fragment, while the *CD1A* and *CD1D* probes both hybridized to an ~160/190 kb doublet. The bigger fragment (i.e. 190 kb) was probably due to an incomplete digest. Based on these results and taking into consideration the products of partial digestion, a long-range restriction map at CD1 locus was constructed as shown in Figure 3.

Cosmid cloning and walking

As cosmid clones allow DNA inserts between 30 and 45 kb, it seemed probable that some, if not all, of the CD1 genes could be linked by individual cosmids. The α3 domains of the five CD1 genes share high sequence identity (73−88%) such that a DNA probe covering the *CD1A*-α3 exon cross-hybridizes to all CD1 genes. Each of the CD1 genes gives a characteristic *Eco*RI restriction fragment in Southern blot analysis using this cross-hybridizing probe: *CD1A*, 4.5 kb; *CD1B*, 0.9 kb; *CD1C*, 7.2 kb; *CD1D*, 3.1 kb, *CD1E*, 2.6 kb. Thus cosmids were screened using gene-specific probes and then tested for the presence of the characteristic *Eco*RI fragments.

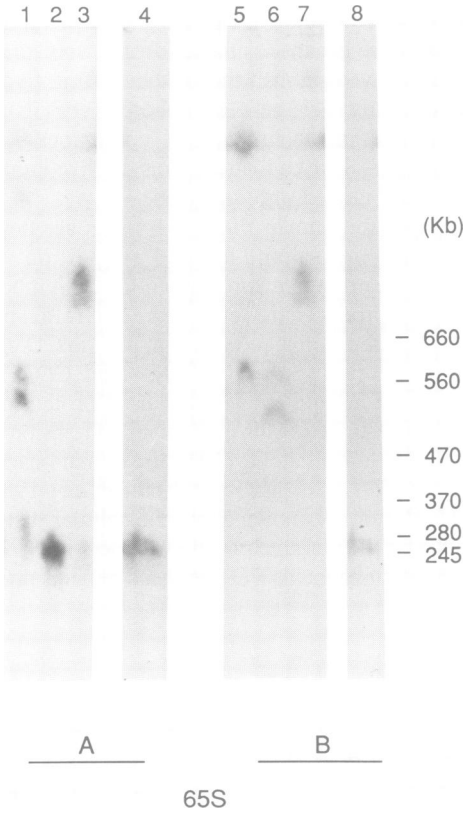

Fig. 1. PFGE of human CD1 genes. Restricted DNA from Molt 4 (NH17) was resolved by PFGE with 65 s pulses to resolve larger DNA fragments between 200 and 1000 kb in size under the conditions described in Materials and methods, blotted to Hybond N membrane and hybridized to various CD1-specific probes. The filter was stripped between each probing. (**A**) The pattern of hybridization when either *CD1A* or *CD1D* probes were used: (**B**) the pattern when either *CD1B* or *CD1C* (or *CD1E* in lane 5) probes were used. Enzyme digests were as follows: **lanes 1** and **5**, *Sal*I; **lanes 2** and **6**, *Sal*I−*Sfi*I; **lanes 3** and **7**, *Sfi*I; **lanes 4** and **8**, *Xho*I.

Linkages of CD1B and CD1E

Cosmid clones for various CD1 genes have been isolated from library A, which was constructed from *Hin*dIII partially digested DNA from a human tumour cell line, Colo 320 HSR

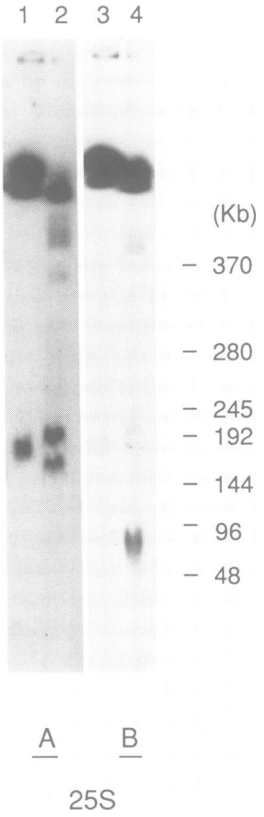

Fig. 2. Splitting of the CD1 complex locus by *Sal*I digest and PFGE. Restricted Molt 4 (NH17) DNA was resolved by PFGE with 25 s pulses to resolve smaller DNA fragments between 50 and 400 kb and processed as described in Figure 1. (**A**) The pattern when *CD1A* or *CD1D* probes were used; (**B**) the pattern when *CD1C* probe was used for hybridization in the Southern blot analysis. **Lanes 1** and **3** were *Sal*I digests; **lanes 2** and **4** were *Sal*I−*Xho*I double digests.

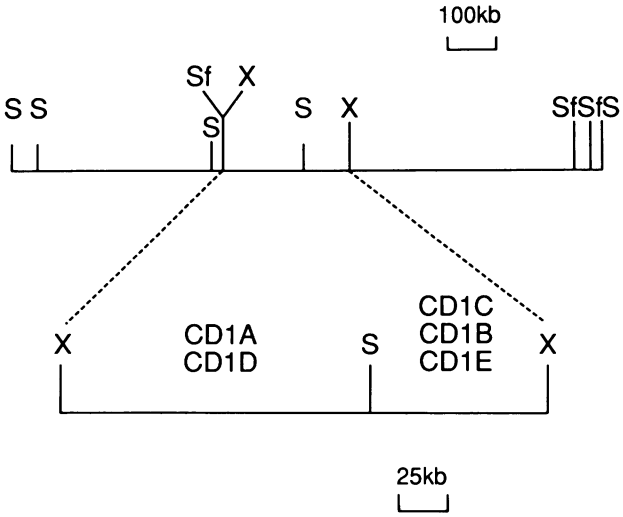

Fig. 3. A partial, long-range restriction map around the human CD1 complex. Restriction sites include only those which can be derived from Figures 1 and 2 and this may not include all sites. The five CD1 genes can be divided into two groups by a *Sal*I digest of the genomic DNA and PFGE. S, *Sal*I; X, *Xho*I; Sf, *Sfi*I.

(Buluwela *et al.*, 1988). These include Y49G for *CD1A*, Y75A for *CD1C*, Y33G for *CD1D* (Figure 4) and Y11LB for *CD1E* (not shown). Each of these cosmid clones contain only one CD1 gene. Screening of the same library with the *CD1B* specific probe gave cosmid Y18A (and cosmid Y19B, not shown) which harbours both the *CD1B* and *CD1E* genes (Figure 4). Further restriction mapping of Y18A (not shown) revealed that the intergenic region between *CD1B* and *CD1E* genes is ~20 kb, and that these two genes are arranged in head-to-head orientation (see below in Figure 6).

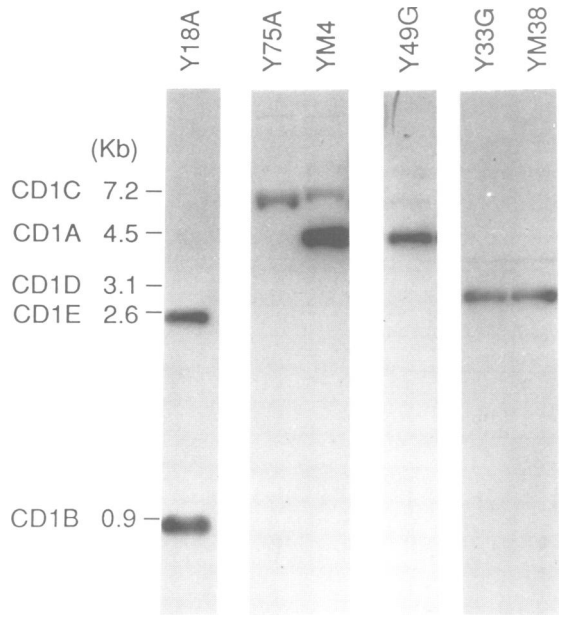

Fig. 4. Southern blot analysis of some cosmid clones for five human CD1 genes. Cosmid clones Y18A, Y75A, Y49G and Y33G were isolated from library A; while clones YM4 and YM38 were isolated from library B. Cosmid DNA was digested with restriction enzyme *Eco*RI, blotted to Hybond N membrane, and hybridized with the (cross-hybridizing) *CD1A* α3 probe to identify the characteristic size *Eco*RI fragments for various CD1 genes.

Linkage of CD1A and CD1C

DNA probes were derived from *CD1A* and *CD1D* cosmid clones in an attempt to link them to other CD1 genes by a cosmid walking procedure. This approach was unsuccessful when library A was used as identical clones were obtained repeatedly in various screenings. Therefore, two new genomic libraries were constructed. Library B was made from *Bam*HI partially digested DNA isolated from Molt 4. Library C was made from *Mbo*I partially digested DNA from a lymphoblastoid cell line, AD (Yu *et al.*, 1986). In this way we hoped to avoid non-random partial digestion of genomic DNA in a particular library.

The linkage of *CD1A* and *CD1C* gene was established by a new cosmid clone, YM4, isolated from library B. This cosmid contains the *CD1A*-specific 4.5 kb, and the CD1C-specific 7.2 kb *Eco*RI fragments (Figure 4). Refined mapping showed that the *CD1A* is ~25 kb upstream to the *CD1C* gene and they are arranged in the same transcriptional orientation (see below in Figure 6).

Further screening of library B with specific probes yielded cosmids YM5 with the *CD1C* gene, and YM38 and YM1 with the *CD1D* gene (see below in Figure 6).

Linkage of CD1B and CD1C

As shown in previous sections, the 5′ end of the *CD1C* gene is linked to the 3′ end of the *CD1A* gene (as they are contiguous and in the same orientation), while the 5′ end of *CD1B* gene is linked to the 5′ end of the *CD1E* gene (as they are contiguous and in head-to-head orientation. Since in addition *CD1B* and *CD1C* are closely linked as they hybridize to the same *Sal*I to *Xho*I restriction fragments (Figure 1), they should be linked through their 3′ ends in tail-to-tail orientation. A 1.8 kb DNA probe that

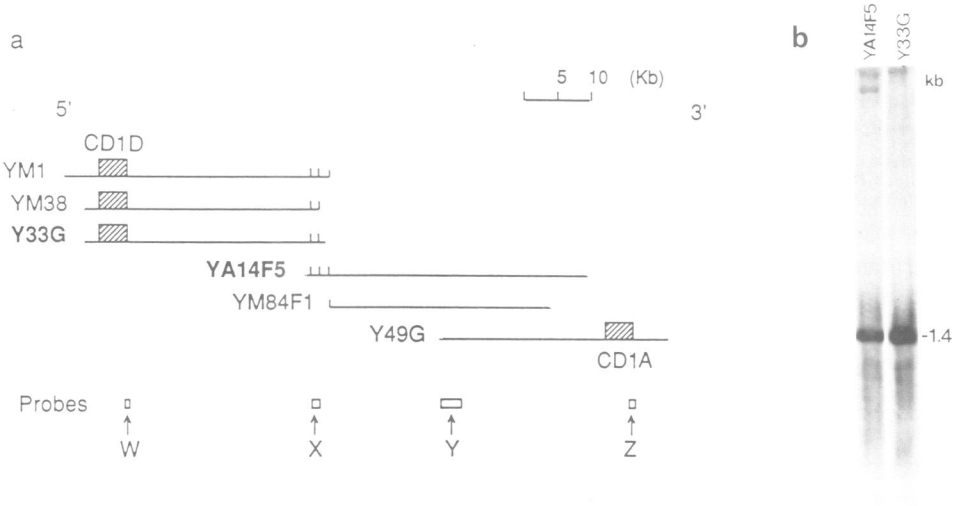

Fig. 5. Linkages of the *CD1D* and *CD1A* genes. (**a**) YM1, YM38 and Y33G were overlapping cosmid clones containing the *CD1D* gene. Y49G contains the *CD1A* gene and extends to its 5′ direction by 25 kb. A unique, 3.2 kb probe (i.e probe Y) derived from the 5′ end of Y49G was used to isolate YM84F1 and YA14F5 from library B and C respectively. The latter shares an 1.4 kb *Bam*HI fragment with Y33G, YM38, and an additional 1.5 kb *Bam*HI fragment with YM1. The *Bam*HI restriction sites giving rise to the 1.4 and 1.5 kb fragments discussed in the text are marked by small vertical lines in the cosmids concerned. (**b**) The 1.4 kb *Bam*HI fragment from Y33G (i.e. probe X) was eluted and used to hybridize *Bam*HI restricted DNA from YA14F5 and Y33G in a Southern blot analysis. Hybridization of this probe to both cosmids at a specific restriction fragment of identical size indicated the two cosmids are linked. W and Z show location of other relevant specific probes.

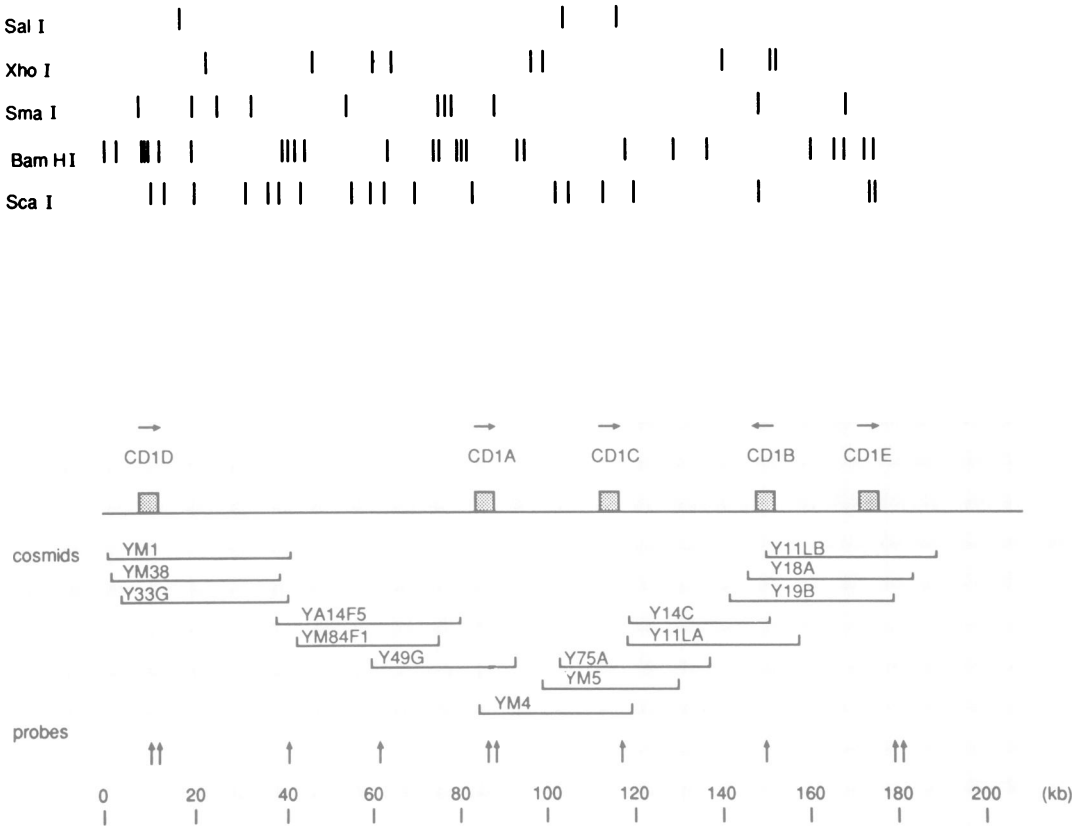

Fig. 6. A molecular map of the human CD1 complex. The five CD1 genes are linked by 14 overlapping cosmids which span 190 kb of DNA. All CD1 genes are arranged in the same transcriptional orientation except CD1B. Complete restriction enzyme maps for *Sal*I, *Xho*I, *Sma*I, *Bam*HI and *Sca*I were deduced by single and double digests of cosmid DNA and the application of CD1-specific and cross-hybridizing probes in Southern blot analyses.

corresponds to the 3′ end of the *CD1B* genomic clone, γR1M5 (Martin *et al.*, 1986) and is ~ 2 kb downstream of the *CD1B* gene (Figure 6), was used to screen library A. This yielded two different clones, Y14C and Y11LA. Both of these clones contain the coding sequence for *CD1B* and extend to the 3′ direction. Southern blot analysis showed that the longer clone, Y11LA, also hybridized to the *CD1C*-specific probe, which is a 0.8 kb *Hin*dIII fragment located at the 3′ end of the *CD1C* gene. Further mapping experiments confirmed this overlap, e.g. the previously isolated Y75A, which includes the *CD1C* gene, shares the same restriction site pattern with Y11LA over 21 kb of DNA. Thus the *CD1B* and *CD1C* genes are ~32 kb apart and are organized in opposite orientations (see below in Figure 6).

Linkage of CD1A and CD1D

Since PFGE data suggested that the last remaining *CD1D* gene is linked to *CD1A*, we concluded that the *CD1D* gene should be located at the 5′ direction of the *CD1A* gene. A non-repetitive probe of 3.2 kb that is a *Hin*dIII−*Sac*I fragment located 25 kb 5′ to the *CD1A* gene from Y49G (i.e. probe Y, Figure 5a), was used to screen the cosmid libraries. Library B yielded YM84F1. This clone further extends to the 5′ direction of Y49G by 17 kb. However, it does not overlap with the *CD1D* clone, Y33G. Library C yielded YA14F5. This clone contains a bigger insert and extends 21 kb of Y49G. It shares with Y33G an 1.4 kb *Bam*HI fragment (not shown). To test whether the 1.4 kb *Bam*HI fragments from both cosmids were identical, they were eluted from agarose gel, and used for further fine restriction mapping. They revealed identical restriction patterns for common cutters such as *Hin*fI, *Mbo*I and *Ava*II (data not shown). In addition they were used as probes in Southern analysis of a *Bam*HI digest of the cosmid clones of YA14F5 and Y33G. A single common component of correct size was obtained (Figure 5b). Further confirmation was obtained from another *CD1D* cosmid clone, YM1, which shares an additional 1.5 kb *Bam*HI fragment with YA14F5 (Figure 5a). Thus it was found that the *CD1D* gene is located 73 kb away from the *CD1A* gene and that these genes are arranged in the same transcriptional orientation. This is the biggest intergenic distance among the five CD1 genes.

A detailed restriction map for *Sal*I, *Xho*I, *Sma*I, *Bam*HI and *Sca*I at the CD1 complex is shown in Figure 6. All CD1 genes are arranged in the same transcriptional orientations, except for *CD1B*. The whole CD1 complex spans ~190 kb in size and has been cloned by 14 overlapping cosmids.

Discussion

The tight linkage of the five human CD1 genes was established by PFGE and then cosmid cloning and walking techniques. Application of gene-specific probes in Southern blot analysis of PFGE DNA from Molt 4 indicated that the CD1 genes cluster in a region <260 kb in size, and that they can be divided into two groups by a *Sal*I digest: *CD1A*

+ *CD1D* and *CD1B* + *CD1C* + *CD1E*. These results encouraged and facilitated the subsequent cosmid cloning and walking experiments to determine the exact arrangements of the CD1 genes with the complex locus by giving the size limits and the direction of walking.

The five CD1 genes have been linked by 14 overlapping cosmids isolated from three different libraries. They are positioned in the following order: *CD1D*−*CD1A*−*CD1C*−*CD1B*−*CD1E*. The intergenic distances are 73, 25, 32 and 20 kb respectively. All CD1 genes are arranged in the same transcriptional orientation except *CD1B*. It is interesting to note that the genes encoding the defined *CD1*a, *-b*, *-c* antigens are flanked by the *CD1D* and *CD1E* genes, which are the more divergent members in the family (Calabi *et al.*, 1989b). These are also the two whose gene products have not yet been identified by antibodies. Recent sequence analyses of nine CD1 genes from human, mouse and rabbit has allowed the categorization of CD1 genes into two classes, i.e. *CD1D* (R3) and 'classic' CD1 (Calabi *et al.*, 1989a,b). It may be significant that the human *CD1D* gene is separated from the classic CD1 gene cluster by a distance of 73 kb, compared with intergenic distances of 20−32 kb within the classic genes. This observation favours the notion that the two classes of genes were the result of an early gene duplication. Subsequent deletion and/or further duplications within each class could have resulted in the variation of gene number in rodents, rabbits and humans.

The MHC-related CD1 genes are located in region q22-23 of chromosome 1 (Albertson *et al.*, 1988). This is the only known example of β2 microgloblin associated gene products that do not map within the human MHC locus at chromosome 6p21.3 (reviewed in Olisen *et al.*, 1987). A rat neonatal receptor for IgG (FcRn) has been sequenced and shown to be distantly related to the MHC class I and to CD1, and to associate with β2 microglobulin (Simister and Mostov, 1989). Thus it would be of particular interest to determine the chromosomal location of the human counterpart of FcRn. Recently it has been found that another gene of the Ig superfamily (reviewed in Williams and Barclay, 1988), the human Blast-1, is located in chromosome 1q21-23 (Staunton *et al.*, 1989). This may also be true for the human genes encoding for the receptors for the Fc portion of IgG, FcγRII (*CD32*) and FcγRIII (*CD16*) (Grundy *et al.*, 1989). An intriguing possibility is that an early translocation had occurred between the precursors of chromosomes 1 and 6 generating two related complexes: the MHC in chromosome 6 and the CD1−Blast 1−(*CD16*−*CD32*, and FcRn?) in chromosome 1. It is also noteworthy that another group of related genes with at least six members and involved in immune function, i.e. the regulator of complement activation (RCA locus), is located at a nearby region, 1q32 (Weis *et al.*, 1987; Carroll *et al.*, 1988).

The chromosomal region where the CD1 genes are mapped was observed to be involved in chromosomal translocation events in certain lymphoblastic leukaemias, e.g. t(1;19) (q23;p13) in some pre-B cell leukemias (Williams *et al.*, 1984; Carroll *et al.*, 1984). A detailed restriction map around the CD1 complex has been constructed in this study. The map may be useful for deriving markers to study the aberrant chromosomal translocational events, and for investigating the relationship between the CD1 genes and these leukaemias.

Materials and methods

Pulse field gel electrophoresis

Cross-field gel electrophoresis apparatus was constructed by the MRC Laboratory of Molecular Biology Workshop according to details provided by Professor E.M.Southern's laboratory (Oxford). Human genomic DNA used for mapping was from a derivative (NH17; Burrone *et al.*, 1983) of a cortical thymocyte cell line Molt 4, which is a high CD1 expressor. Log phase culture cells were embedded in low gelling temperature (LGT) agarose, processed and digested as described in Van Ommen and Verkerk (1986) and Southern *et al.* (1987). Restriction enzyme digested DNA were resolved by PFGE with 65 s pulses at 20°C for 35−40 h, with 0.5 × TAE (20 mM Tris−acetate, 1 mM EDTA, pH 8.5) buffer for fragment size between 200 and 1000 kb, or with 25 s pulses under the same conditions for fragment size between 50 and 400 kb. Gel-resolved DNA was UV irradiated and blotted by Hybond N membrane (Amersham) according to the procedure of Southern (1975) and probed with CD1 gene-specific probes. After each probing, filters were stripped twice by 0.1 × SSC, 0.5% SDS, 20 min at 95°C before hybridization with the next probe.

Probes

The *CD1A*-specific probe was a 250 bp *Sty*I−*Eco*RV fragment derived from a cDNA clone, FCB (Calabi and Milstein, 1986). This fragment is in the 3′ untranslated region of *CD1A* (i.e. probe Z, Figure 5a). The CD1B-specific probe was a ~500 bp fragment from an M13 shotgun subclone, L5M4, which corresponds to the 3′ untranslated region of a cDNA clone (Martin *et al.*, 1987). the *CD1C*-specific probe was a 0.8 kb *Hin*dIII fragment located at the 3′ end of the CD1 gene of a λ clone, R7L4 (Martin *et al.*, 1987). The specific probe (i.e. probe W in Figure 5a) was two ~200 bp *Dra*I fragments which are located at the 3′ untranslated region of a cDNA clone, FCC (Calabi *et al.*, 1989b). The CD1E probe was a ~400 bp *Eco*RI fragment located after the α3 exon (Calabi *et al.*, 1989b). In some early experiments a 2.6 kb *Eco*RI fragment covering the α1−α3 domains of the R2 gene was used. The CD1 cross-hybridizing probe is a ~400 bp *Sty*I fragment of the FCB clone, which corresponds to the α3 domain of *CD1A*. Newly derived DNA probes for cosmid walking were tested to ensure that they were not repetitive in nature by Southern blot analysis with radioactive, sonicated, human genomic DNA. DNA probes were labelled with either Amersham or Pharmacia multi-prime labelling/oligolabelling kits.

Construction and screening of cosmid libraries

Cosmid clones were isolated from three different human genomic libraries. Library A was a gift from Dr T.H.Rabbitts' laboratory. It was constructed using *Hin*dIII partially digested DNA isolated from a tumour cell line, Colo 320 HSR (Buluwela *et al.*, 1988). The cosmid vector was Lorist 6 (Gibson *et al.*, 1987). Library B was constructed with *Bam*HI partially digested DNA from Molt 4. Library C was made with *Mbo*I partially digested DNA from a lymphoblastoid cell line AD (Yu *et al.*, 1986). Digested DNA was fractionated (33−45 kb) twice with either sucrose gradients or LGT agarose gel electrophoresis. Both libraries B and C used *Bam*HI cleaved, phosphatased Lorist 6 as cloning vector. Packaging extracts were from Strategene (Gigagold). For libraries A and B, 0.5−1.5 × 10^6 colonies were screened. Only ~2 × 10^5 clones were screened in the case of library C. Screening and rescreening of positive clones were essentially as described by Little (1987).

Restriction mapping of cosmid clones

Restriction maps of *Sal*I, *Xho*I, *Sma*I, *Bam*HI and *Sca*I of various cosmid clones were deduced from digested DNA resolved by standard agarose gel electrophoresis (Maniatis *et al.*, 1982) and Southern blot analysis using a cross-hybridizing CD1 probe and/or various specific probes as described in the text.

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