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Linkage map of the human major histocompatibility complex including the tumor necrosis factor genes

(pulsed-field gel electrophoresis/human leukocyte antigen/restriction map/complement genes/human genome)

MICHAEL C. CARROLL*, PHILIP KATZMAN*, ELIZABETH M. ALICOT*, BEVERLY H. KOLLER†, DANIEL E. GERAGHTY†, HARRY T. ORR†, JACK L. STROMINGER‡§, AND THOMAS SPIES‡

*Departments of Pediatrics and Biological Chemistry, Harvard Medical School, Boston, MA 02115; †Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN 55455; ‡Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138; and §Dana–Farber Cancer Institute, Boston, MA 02115

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ABSTRACT **The tumor necrosis factor (TNF) α and β gene pair has been linked in the human major histocompatibility complex to HLA-B, HLA-C, and, tentatively, HLA-E and HLA-A on one side and to the class III complement/steroid 21-hydroxylase gene cluster on the other by pulsed-field gel electrophoresis. The TNF genes are located 200 kilobases (kb) centromeric of HLA-B and about 350 kb telomeric of the class III cluster. Together with previous data on the linkage and structures of the class II and class III regions, a restriction map of the entire human major histocompatibility complex of about 3500 kb has been prepared.**

The human major histocompatibility complex (MHC) is located between 6p21.1 and 6p21.3 (1) and includes several gene families clustered in distinct regions. Class I and class II genes encode cell-surface glycoproteins that are required for recognition of foreign antigens by cytolytic and helper T lymphocytes (for reviews, see refs. 2 and 3). The class III genes encode the complement factors C2, Bf, C4A, and C4B and two steroid 21-hydroxylase (21-OHase A and 21-OHase B) genes (4–6). In addition, the closely linked tumor necrosis factor α and β genes (TNFα and TNFβ) (7) have also been shown to be included in the human MHC (8). Analysis of a panel of MHC deletion mutant cell lines by Southern blot hybridization indicated that the TNF genes are located either at the centromeric end of the class II region close to DP or between DR and HLA-A. These results were corroborated by molecular mapping within the H-2 complex of the mouse (9, 10). The TNF genes were found 70 kilobases (kb) centromeric of the H-2D locus, the murine homolog of HLA-B (9). Thus, a location of the TNF genes in proximity of HLA-B in man seemed most likely.

To establish the genetic linkage in man and to study the physical organization of genes throughout the human MHC, pulsed-field gel electrophoresis (PFGE) (11, 12) combined with Southern blot hybridization (13) was applied. This technique has been used to determine the arrangement of the class II genes within a region of 900 kb (14) and to provide a version of the whole MHC (15) slightly different from that presented here and not including the TNF genes. In addition, the class II and class III regions were linked within a common 1000-kb *Not* I restriction fragment (15, 16). These studies suggested, on the basis of a linear alignment of restriction fragments hybridizing with class II, class III, or class I coding sequence probes, that the entire MHC spans about 2000–3000 kb. However, the class III and class I regions were not linked and restriction fragments were not overlapped to confirm contiguous sites. In the present report, data that link the complement/21-OHase, TNFα and -β, and HLA-B, -C, -E, and -A genes are presented, thus connecting the class I and class III regions. In addition, the location of the TNF genes in the proximity of HLA-B and their relative orientation are determined.

MATERIALS AND METHODS

High molecular weight genomic DNA was prepared, digested with restriction endonucleases, and fractionated in agarose gels using an orthogonal-field alternation gel electrophoresis (OFAGE) apparatus (12, 17) with several modifications (18). The OFAGE unit was constructed by the biological chemistry workshop at Harvard Medical School. The source of DNA was an Epstein–Barr virus-transformed B-cell line derived from peripheral blood cells of a consanguineous HLA homozygous Icelandic individual (186-2137) typed as A2, B7, DR2, C4A 3, C4B QO, Bf S, C2 C.

RESULTS AND DISCUSSION

The probes employed and a linkage map of the human MHC derived from the data presented below are shown in Fig. 1.

Linkage of TNFα and -β Genes to HLA-B. The TNFα and -β genes have been linked to HLA-B by PFGE (see below) and in a series of overlapping cosmids covering 300 kb (T.S., unpublished data). PFGE was used to link the TNFα and -β genes to HLA-B by demonstrating that cosmid "walking" probes flanking these genes were linked. Cosmid probe X is 25 kb 3′ of the TNFα gene, whereas probe Y is 105 kb 5′ of TNFβ. Probe Z is 35 kb 5′ of HLA-B (and now also known to be 175 kb 5′ of TNFβ). Genomic probes Y and Z, which represent the regions flanking the TNFβ and HLA-B genes, respectively, were linked on a common 325-kb *Bss*HII fragment along with TNFα and probe X (Fig. 2 *B–D*, lanes 1). However, the X and TNFα probes were located on the same 225-kb *Mlu* I fragment (Fig. 2 *B* and *C*, lanes 4), whereas probe Z hybridized to two smaller fragments of 150 kb shared with probe Y and 125 kb (Fig. 2*D*, lane 4). Moreover, after *Sal* I digestion probe Y (which flanks TNFβ) was included in a 450-kb fragment together with the Z and HLA-B and -C probes (Fig. 3 *B–D*, lanes 1; data not shown for HLA-C). Probe X and TNFα, however, hybridized to a distinct 275-kb *Sal* I fragment (Fig. 1). Thus, the TNF and HLA-B and -C genes were linked by PFGE. The minimum distance separating the TNF and HLA-B genes was estimated as 150 kb since neither of these was included in the 150-kb *Mlu* I fragment containing the Y and Z probes (Fig. 2*D*, lane 4; Fig. 1). The hybridization of probe Z to an additional 125-kb *Mlu*

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Abbreviations: MHC, major histocompatibility complex; TNF, tumor necrosis factor; PFGE, pulsed-field gel electrophoresis; 21-OHase, steroid 21-hydroxylase.

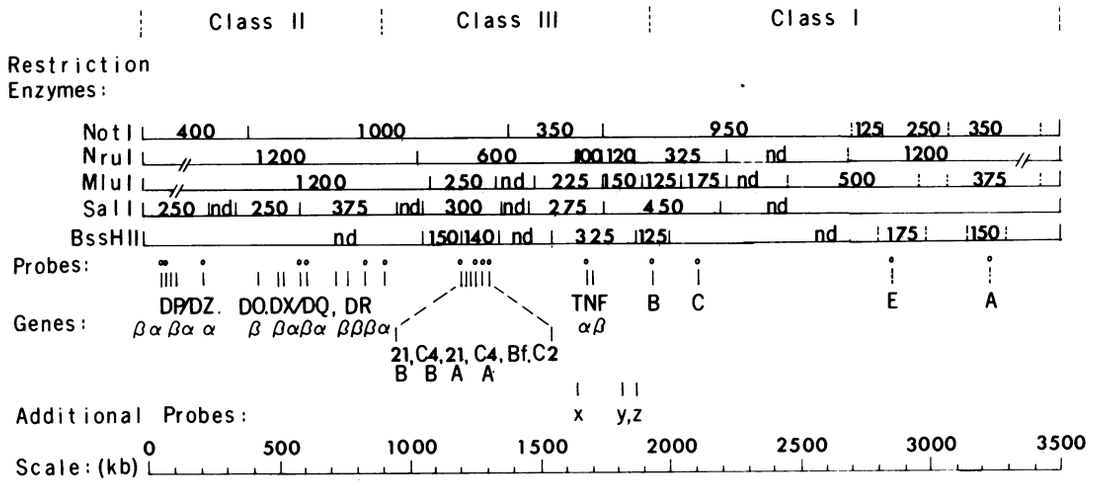

FIG. 1. Organization of genes within the human MHC. The restriction map was prepared by PFGE and Southern blot analysis using five different restriction endonucleases and DNA probes (indicated on the map by a circle over a vertical bar) from each of the three subregions as follows: DRβ and DQβ cDNA (19), DQα cDNA, DPα and DZα genomic DNA (20), DRα cDNA (21), DPβ cDNA (22), C4 cDNA (23), Bf and C2 cDNA (24), HLA-B cDNA (25), flanking region of the HLA-C gene (ref. 26; H.T.O., unpublished data), 3' untranslated region of the HLA-A2 gene (27), flanking region of the HLA-E gene (ref. 26; H.T.O., unpublished data), and TNFα cDNA (28). Approximate positions of genes and of additional 200- to 300-base-pair (bp) genomic single-copy probes X, Y, and Z derived from a contiguous TNF-HLA-B cosmid cluster are depicted by vertical bars. Restriction fragments were aligned on the basis of single and double digests and sequential hybridization of Southern blots with the respective DNA probes. However, small gaps could exist between apparently contiguous fragments since fragments <50 kb would be missed in the 30- to 60-sec pulsed gels. Fragment sizes are estimates within 10%. Some digests may not have gone to completion, in part due to methylation, and thus some restriction sites may not be designated. Regions where contiguous overlapping restriction fragments were not identified are indicated by "nd" (not determined). Note that the map indicates a normal haplotype with two genes each for C4 and 21-OHase, although the haplotype of the cell line used in this study has a deletion that includes both the C4B and 21-OHase A genes.

I fragment indicated either a duplication of the 200-bp Z sequence or its split by a *Mlu* I cleavage site.

The orientation of the TNFα and -β genes, which are in tandem 3' → 5' orientation separated by 1.2 kb (7), was determined by finding that the Y probe (5' to TNFβ) was linked to HLA-B (Figs. 2 and 3), whereas the X probe (3' to TNFα) was linked to the class III complement/21-OHase genes (see below). The Y probe was linked to probe Z by the *Sal* I digest as were the X and TNF probes (see above). Probe X and TNF were also linked on a 350-kb *Not* I fragment as were probe Y and HLA-B and -C on a 950-kb fragment. Since probe Z was found on a 325-kb *Bss*HII fragment with TNF and probe Y, whereas HLA-B was on a separate fragment (Fig. 1), probe Z must be located between probe Y and HLA-B. Thus, within this region these three genes are in the order TNFα (3' → 5'), TNFβ (3' → 5'), and HLA-B (5' → 3' in the opposite orientation). The PFGE results agree with the molecular map of the 300-kb cosmid cluster where the 5' end of the HLA-B gene was 210 kb upstream of the 5' end of TNFβ (T.S., unpublished data).

Linkage of TNF Genes to the Class III Gene Cluster. To explore the linkage of the TNF and complement/21-OHase genes, Southern blots of PFGE gels with *Not* I- and *Nru* I-digested DNA samples were hybridized with class III C4, X, and TNFα probes. A 600-kb *Nru* I fragment contained C4 and probe X but excluded TNFα, which was found on the adjacent 100-kb fragment (Fig. 4 *B–D*, lanes 2; Fig. 1). However, C4 and X probes mapped to different *Not* I fragments of 1000 and 350 kb, respectively (Fig. 4 *B* and *D*, lanes 6). The 350-kb *Not* I fragment was not found in a previous study in which TNF probes were not employed (15). Similarly, the 250- and 125-kb *Mlu* I fragments (containing C4 and HLA-B, respectively) are not contiguous but are separated by at least 500 kb. To confirm that the 600-kb *Nru* I fragment was common for the C4 and X probes, a *Nru* I/*Not* I double digest was analyzed. As predicted, the resulting 350- and 250-kb fragments hybridized to the C4 and X probes, respectively (Fig. 4 *B* and *D*, lanes 5). Thus, the TNF and complement/21-OHase genes were physically linked. The

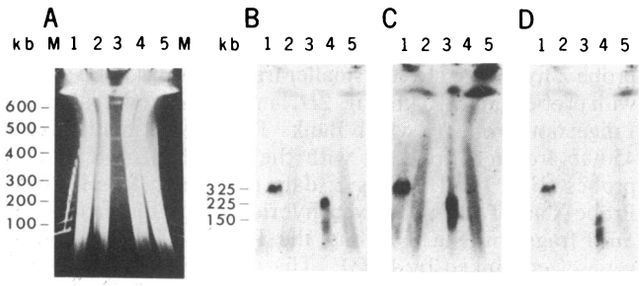

FIG. 2. Linkage of TNFα and HLA-B by PFGE and Southern blot analysis using probes X, TNFα, and Z. Current was switched each 30 sec for 18 hr. (*A*) Ethidium bromide-stained gel with phage λ oligomer (lanes M) and yeast (*Saccharomyces cerevisiae*) chromosomes (lane 3) as markers. (*B–D*) The same filter, stripped between hybridizations with the X, TNFα, and Z probes, respectively. Lanes 1, *Bss*HII; 2, *Cla* I; 3, yeast marker; 4, *Mlu* I; 5, *Sac* II. Digests with *Cla* I and *Sac* II did not yield discrete bands.

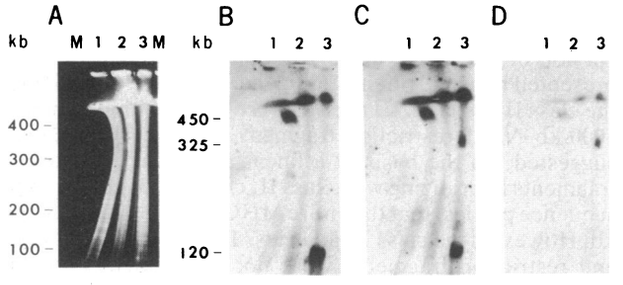

FIG. 3. Linkage of the TNF and HLA-B genes by PFGE and Southern analysis using probes Y, Z, and HLA-B. Current was switched each 15 sec for 20 hr. (*A*) Ethidium bromide-stained gel. (*B–D*) The same filter, stripped between hybridizations with the Y, Z, and HLA-B probes, respectively. Lanes M, as in Fig. 2. Lanes 1, *Sal* I; 2, *Not* I; 3, *Nru* I/*Not* I.

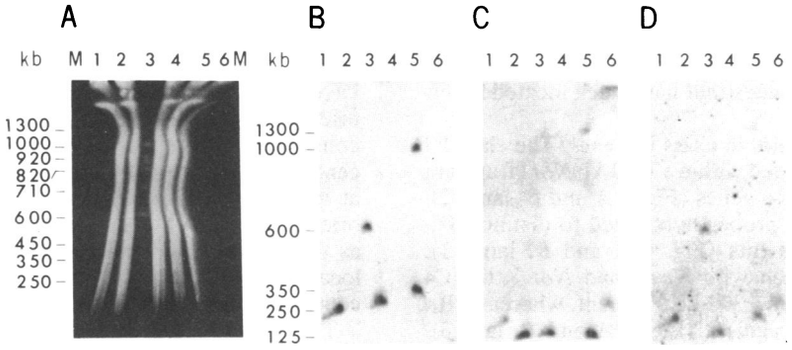

FIG. 4. Linkage of the complement/21-OHase and TNFα genes by PFGE and Southern blot analysis using probes C4, TNFα, and X. Current was switched each 60 sec for 20 hr. (*A*) Ethidium bromide-stained gel. The order of lanes is reversed from that shown in *B–D*. (*B–D*) The same filter, stripped between hybridizations with the C4, TNFα, and X probes, respectively. Lanes M, as in Fig. 2. Lanes 1, *Mlu* I; 2, *Nru* I; 3, *Nru* I/*Mlu* I; 4, yeast; 5, *Nru* I/*Not* I; 6, *Not* I.

minimum distance separating these loci is 250 kb (Fig. 1). Therefore, the region between the class III and the HLA-B genes spans at least 400 kb. The general organization of these genes in the mouse, including C4, TNFα and -β, and H-2D corresponds (29), although, as in man, they have not been linked by cosmid cloning. The order of the genes within the complement/21-OHase cluster has been determined (4–6). The orientation of this cluster with respect to the class II and TNF genes (i.e., whether C2, for example, is closest to *E*α or to TNFα) was obtained in a study of the corresponding mouse genes (29) and is based on the assumption of the homologous order in man. We have not independently derived the orientation of the class III region gene cluster within the human MHC. It should be noted, however, that the proposed order in the mouse (29) is based on a restriction map of a *Pvu* I fragment containing the entire class III cluster and *E*α, the most telomeric gene in the class II subregion (and a similar *Mlu* I fragment reported in a footnote), which apparently linked 21-OHase B at one end of the cluster with *E*α. A restriction site *within* the class III region (and thus separating its two ends) suitable for PFGE analysis has not been found in either mouse or man. Moreover, the presumed reciprocal linkage of C2 at the other end of the cluster to TNF genes was not obtained. Thus, further confirmation of the proposed order may be desirable.

Linkage of HLA-B to the HLA-C, HLA-E, and HLA-A Genes. Within the class I region, the HLA-B and -C genes were included in common 950-, 325-, and 450-kb *Not* I, *Nru* I, and *Sal* I fragments but were localized to different 125- and 175-kb *Mlu* I fragments, respectively (Figs. 1 and 5 *B* and *C*, lanes 1, 2, and 4). The map derived from these data indicated a maximum distance of 250 kb between HLA-B and -C since both probes hybridized to an *Sfi* I fragment of that size (Fig. 5 *B* and *C*, lanes 8). The linkage has not yet been definitively extended telomeric to HLA-A. However, the HLA-E and -A genes were mapped to distinct *Not* I fragments of 250 and 350 kb, respectively, but to a common *Nru* I fragment of 1200 kb (Fig. 1). An additional 375-kb partial *Not* I fragment, as well as the 250-kb fragment, hybridized to a probe for the HLA-E gene. The order of the two genes with respect to HLA-C was based on analysis of deletion mutants (ref. 26; B.H.K., D.E.G., and H.T.O., unpublished data). Although the position of the two genes was tentative, an estimate of the minimum distance between HLA-E and -C would be 650 kb based on the analysis with *Not* I. The distances between HLA-C, -E, and -A can only be estimated but the total length of the class I region is a minimum of 1600 kb from the sizes of the *Not* I fragments.

The HLA-E gene is almost certainly identical to the novel class I gene contained in clone RS5 (30). The position of the *Not* I fragment containing this gene was placed telomeric to HLA-A (15) because of genetic information reported earlier (31). However, a more recent analysis of the position of HLA-E employing deletion mutants indicates that this gene lies centromeric to HLA-A (B.H.K., D.E.G., and H.T.O., unpublished data). Thus, the 250- and 125-kb *Not* I fragments (which comprise the 375-kb partial *Not* I fragment) must lie between the 950- and 350-kb *Not* I fragment as shown in Fig. 1. The present data also cannot exclude the occurrence of additional relatively small *Not* I fragments within this region, not detected by the probes employed (Fig. 1). Provided that no additional *Not* I fragments are found, the minimum distance separating HLA-C and -A is about 1000 kb. The present map of the class I region telomeric to HLA-C should

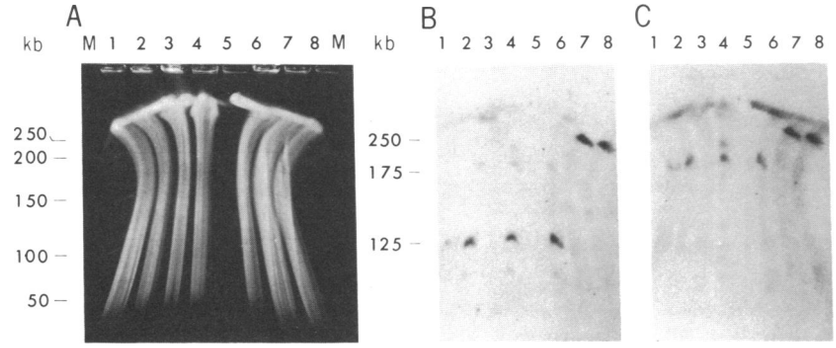

FIG. 5. Linkage of HLA-B and HLA-C by PFGE and Southern analysis using probes HLA-B and -C. Current was switched each 7 sec for 18 hr. (*A*) Ethidium bromide-stained gel. (*B* and *C*) The same filter, stripped between hybridizations with the HLA-B and -C probes, respectively. Lanes M, as in Fig. 2. Lanes 1, *Mlu* I; 2, *Mlu* I; 3, *Nru* I; 4, *Nru* I/*Mlu* I; 5, yeast; 6, *Mlu* I/*Not* I; 7, *Sfi* I/*Not* I; 8, *Sfi* I. Although the HLA-B cDNA probe was specific at high-stringency wash, a faint cross-hybridization with HLA-C was seen (*C*, lanes 1, 2, and 4).

be regarded as tentative, particularly since overlapping clones for this region are not reported and further work is required in any event to establish the position of additional class I genes and pseudogenes that have been located in this subregion (26).

Linkage of Class III Genes to Class II Genes. The class III and class II regions are linked within a 1000-kb *Not* I fragment containing the C4 and DRα genes (Fig. 6 *A* and *B*, lanes 2), as reported (15, 16). The probes hybridized to distinct 600- and 1200-kb *Nru* I fragments (Fig. 6 *A* and *B*, lanes 1). Following double digestion with *Nru* I and *Not* I, the C4 probe was contained within a 300-kb fragment, whereas DRα hybridized to a 700-kb fragment (Fig. 6 *A* and *B*, lane 3). These data illustrate the linkage of the class II and III regions. The precise order of the class II genes in the human MHC was established in an earlier study (14) and, again, has not been independently determined in the present study. Our data in general are in accord with these earlier studies for three of the enzymes used in common—i.e., *Not* I, *Mlu* I, and *Sal* I—except that previous work did not include the DP/DZ cluster on the large *Mlu* I fragment (>1000 kb). However, one study found the class II loci distributed on three distinct *Mlu* I fragments that totaled about 900 kb (15). This discrepancy could be accounted for either by actual genetic differences between the DNA used or by cell-specific differences in methylation. Comparison of the results from *Not* I digests with those published showed a similar pattern with DP/DZ located on a distinct fragment ranging in size from 320 to 600 kb and the remaining class II and complement/21-OHase genes included on a single large fragment of 920–1000 kb (14–16).

Map of the Human MHC. Fig. 1 presents a PFGE linkage map of the entire human MHC of about 3500 kb, completed so far as the data presently available allow. The human MHC is thus about 75% larger than the murine MHC. It contains 50 genes at most, although it is about the same size as the entire *Escherichia coli* genome, which encodes 5,000–10,000 genes. The physical distances determined by PFGE agree roughly with those estimates based on genetic recombination assuming 1000 kb = 1 centimorgan (cM). The overall human MHC has been estimated to be about 3 cM in size and the three regions—i.e., class II, class III, and class I—are estimated to be about 1.2, 1.0, and 0.8 cM, respectively (32). The overall size of the human MHC is larger than the minimum estimate of 2000 kb made for the mouse, where the three regions—i.e., H-2 K/I, H-2 S, and TNF/H-2 D/Qa/T1—were estimated as 600, 900, and 500 kb, respectively. Although the size of the gap between the H-2S region and TNF was not determined, a minimum estimate of 600 kb was proposed (29). The most significant difference in size was between the class I regions

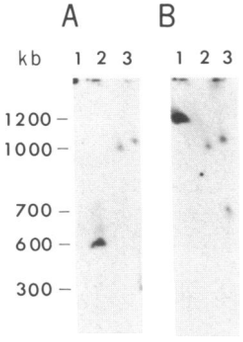

FIG. 6. Linkage of complement/21-OHase cosmid cluster with DRα gene by PFGE and Southern analysis using the probes for C4 and DRα. Current was switched each 60 sec for 20 hr. (*A* and *B*) The same filter, stripped between hybridizations with the C4 and DRα probes. respectively. Lanes 1, *Nru* I; 2, *Not* I; 3, *Nru* I/*Not* I.

(HLA-B/HLA-C/HLA-E/HLA-A and H-2D/Qa/T1) where the homologous region in the mouse was about one-third that of man, despite the fact that genes homologous to Qa and T1 have not yet been found in man. With respect to position and orientation of genes, the H-2 and HLA regions are relatively conserved except for the position of the H-2K locus centromeric to the I region. This translocation accounts for at least some of the apparent size difference. Though the orientation of TNF and HLA-B genes in man was the same as the murine TNF and H-2D genes, the TNF genes were located about 200 kb centromeric of HLA-B but only 70 kb centromeric of H2-D.

The genetic linkage of the TNF and HLA-B loci is extremely interesting with respect to the strong association of the HLA-B27 allotype with ankylosing spondylitis (ref. 33; for a review, see ref. 34). This disease is characterized by an impairment of the sacroiliac joints. Indeed, some biological properties of TNFα and -β suggest that these cytokines could be involved in the destruction of cartilage (35–37). Thus, the possibility that TNFα and/or -β may occur most frequently in some HLA-B27 haplotypes as alleles predisposing to susceptibility to ankylosing spondylitis must be carefully explored.

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