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Linkage of TNF genes to the HLA-B locus

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ABSTRACT

Pulsed field gel electrophoresis was used to determine the location of the tumour necrosis factor (TNF) α and β genes. They were shown to be linked to the HLA-B locus; analagous to their location in mouse, between the complement (class III) region and H-2D. However, the distance between the TNF genes and the class I region was much greater in man, namely about 260 kb, compared to 70 kb in the mouse. This finding may have implications for some HLA associated diseases.

INTRODUCTION

Tumour necrosis factor (TNF) α and β (identical to cachectin and lymphotoxin, respectively) are structurally related cytokines secreted by macrophages and lymphocytes, respectively (1,2). They have cytostatic and cytotoxic effects on certain tumour and virally infected cells, underlining their importance in the immune response (3,4). The TNF α and β genes are separated by 1-2 kilobases (kb) of DNA (5,7). In the mouse, they are located in the major histocompatibility complex (MHC) on chromosome 17 (5). Recently, Müller et al. mapped them more precisely, between the complement (class III) gene region and one of the class I genes, H-2D, 70 kb away from H-2D (9,10). They were also reported to be on the short arm of human chromosome 6, in the vicinity of the MHC, although their precise position was not determined (6). In this paper we describe results of pulsed field gel electrophoresis (PFGE) experiments which show that the human genes are linked to the HLA-B locus, analagous to their position in the mouse.

MATERIALS AND METHODS

Isolation of Cosmid Genomic Clones

The human TNFα gene was isolated from a cosmid gene library constructed from the HLA homozygous B cell line AKIBA [DR2 Dw12](11). Approximately 1 x 10^5 clones were screened by colony hybridization (12) using a ^{32}P-labelled TNF

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α probe made from a 2.9 kb EcoRl subclone derived from a genomic λ-phage insert (13).

PFGE Analysis

High molecular weight DNA was prepared in agarose blocks as described before (14). Approximately 5 x 10^5 cells were encapsulated in each block. The DNA was cut with 40 units of enzyme for 6 - 16h and the blocks were loaded into the slots of 1.0% agarose gels and electrophoresed at 330V in a PFGE apparatus (LKB Pulsaphor). The pulse time was 60 s and the buffer temperature was maintained at 12°C during the run. The DNA on the gel was exposed to UV light (305 nm) for 5 minutes after ethidium bromide staining. Southern transfer to Hybond-N was as recommended by the supplier (Amersham). DNA probes were radiolabelled by the random priming method (15) to a specific activity of about 5 x 10^8 c.p.m. per μg DNA. The filters were hybridized in 6 x SSC (1 x SSC is 0.15M Nacl/0.015M Nacl/0.015M sodium citrate) containing 5 x Denhardt's solution (1 x Denhardt's solution is 0.02% bovine serum albumin/0.02% Ficoll /0.02% polyvinyl pyrrolidone) 10% (wt/vol) dextran sulphate 0.1% NaDodS04(SDS) and salmon sperm DNA (50 μg/ml) for 16hr at 65°C. and washed in 0.2 x SSC at 65°C and exposed to Kodak XAR-5 film for 1-3 days. The filters were stripped of radioactivity by treatment in 0.4M NaOH for 30 mins at 45°C and then in 0.2M Tris-HCl pH 7.5, 0.1 x SSC and 0.1% SDS under the same conditions.

DNA Probes for PFGE Analysis

The TNFα probe was described above. The HLA-B locus specific probe was the 425 PvuII fragment encoding the 3' untranslated region from the HLA-B

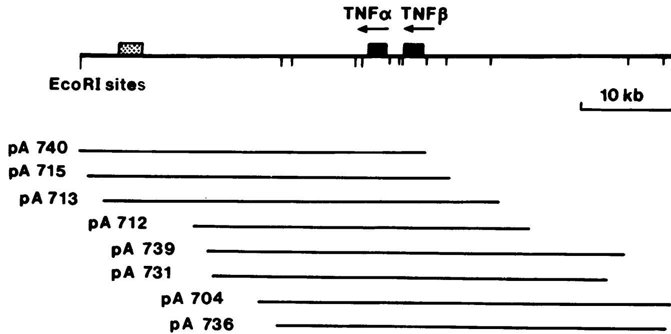

Figure 1. Molecular map of a segment of the human genome containing the TNF genes defined by 8 overlapping cosmid clones. These cosmid clones were isolated from an AKIBA (DR2) genomic library using the TNF α gene probe. Arrows indicate gene orientation (5'→3') which was taken from published data (6,8). The stippled box represents the position of a sequence that hybridized to the TNFα probe under non-stringent conditions.

cDNA, pHLA2 (16-18). A 5′ probe was also used and consisted of a 900 bp EcoRl -PstI fragment from the HLA-B genomic clone, cos30 (courtesy of Dr P.A. Biro) located 30kp upstream of the HLA-B gene.

RESULTS

Isolation of cosmid clones containing TNF genes

Initially, we isolated human TNF genes from the genomic cosmid library to see whether class I related genes were present. 8 overlapping cosmid clones were isolated by colony hybridization with a TNFα probe. (13) They defined a region of 64kb in length around the TNF genes, as shown in Fig. 1, confirming the published map (6,8). No class I related sequences were found on these

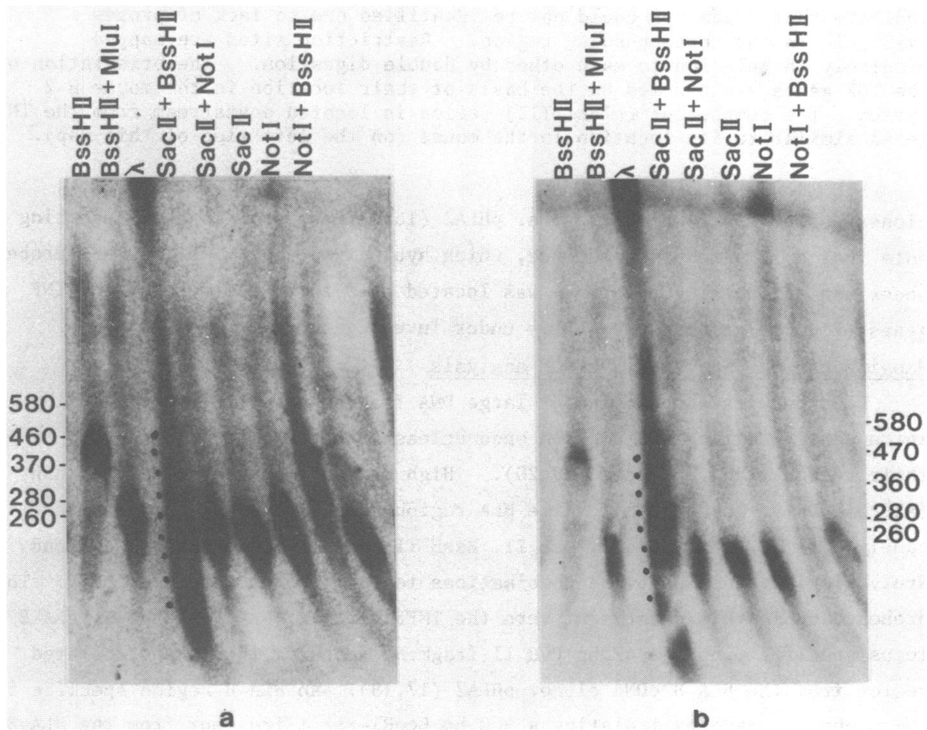

Figure 2. Southern hybridization with PFGE-separated DNA. The autoradiograms show the hybridization of the TNF α gene probe (a) and the HLA-B region specific probe (30kb upstream from HLA-B) (b). The black dots show the positions of multimers of phage λCI857S7 which has a unit size of 48.5kb, visualised by including a small quantity of ^{32}P-labelled λDNA in the hybridisation. Yeast chromosome separated in the tracks at the both ends of the gel provided additional markers (sizes in kb are shown).

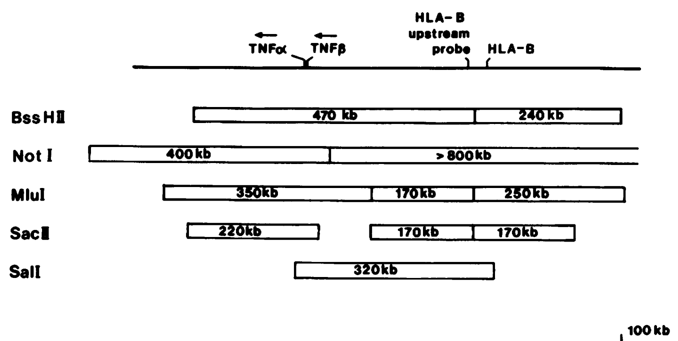

Figure 3. Long range restriction map of the TNF and HLA-B gene region. Fragments obtained with each enzyme are illustrated to scale below the TNFα and HLA-B genes with which hybridisation has been demonstrated. Open spaces indicate that fragments could not be identified due to lack of probes available in the corresponding region. Restriction sites are mapped precisely in relation to each other by double digestion. The orientation of the TNF genes was assumed on the basis of their location in the mouse H-2 system. The complement (class III) region is located downstream from the TNF genes similar to its location in the mouse (on the left side of this map).

clones, using an HLA-B cDNA probe, pHLA2 (16). However, it was interesting to note that a TNFα related sequence, which hybridized weakly to the TNFα probe under non-stringent conditions, was located over 20 kb upstream of the TNF genes (Fig.1). This sequence is under investigation.

Mapping of the TNF genes by PFGE analysis

PFGE permits the mapping of large DNA fragments (up to 2000 kb) using infrequently cutting restriction endonucleases that usually include CpG in their recognition sequences (19,20). High MW DNA from the cell line Mann (A29 B44 DR7), homozygous for the HLA region, was cleaved with 10 enzymes, namely: Sfi I, Cla I, Sac I, Sac II, BssH II, Nar I, MluI, NotI, Nae I and NruI, singly and in pairwise combinations to construct a molecular map. The probes used in this experiment were the TNFα genomic DNA probe and an HLA-B locus specific probe (a 425bp Pvu II fragment encoding the 3′ untranslated region from the HLA-B cDNA clone, pHLA2 (17,18). An HLA-B region specific 5′ end probe was made by isolating a 900 bp EcoR1-Pst I fragment from the HLA-B genomic clone cos30. This fragment was about 30kb away from the 5′ end of the HLA-B gene. Digested DNAs, electrophoresed and blotted onto nylon membranes, were hybridized sequentially with these 3 probes.

Figure 2 shows the results of a typical experiment with the TNFα (a) and HLA-B upstream probes (b). BssH II digestion (lane 1) gave a band of 470 kb

which was positive with both probes. The 3′ end HLA-B probe gave rise to a different band of 240 kb. This result places the TNF genes to the 5′ (upstream) end of HLA-B. Sal I digestion confirmed the TNF α and HLA-B linkage because the 320 kb Sal I fragment was shared by all three probes.

A more detailed restriction enzyme map of the TNF and HLA-B region was constructed as shown in Figure 3. From this map, the distance between the TNF and HLA-B genes was calculated to be approximately 260 kb.

Discussion

The results of the experiments described in this paper show that the human TNF genes are located about 250 kb upstream of the HLA-B gene, presumably between HLA-B and the class III region, by anology with the mouse MHC (10). The difference in distance between the genes in the two species may be related to the fact that the H-2K class I gene is anomolously placed in mouse, relative to other species (20).

The linkage of the TNF genes to HLA-B raises the possibility that some of the diseases such as ankylosing spondylitis (22) and psoriasis vulgaris (23), associated with various HLA-B alleles, or indeed other HLA associated diseases, could be due to linkage with variations in TNF genes. We are searching for DNA polymorphism with TNF probes in order to test this hypothesis.

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