Nycodenz Monocytes (Nyegaard). The monocytes were treated with CAMPATH 1 monoclonal antibody[15] and autologous serum to deplete any residual lymphocytes before exposure to IL-2. In addition, during the cytotoxicity assay, some wells contained MHM23 monoclonal antibody which reacts with the β p95 chain of the LFA-1 molecule and is known to inhibit cytotoxic T cells and NK cells[19,20] (Fig. 2). Neither the treatment with CAMPATH 1 monoclonal antibody, nor the presence of MHM23 monoclonal antibody, nor the combination of both influenced the cytotoxic activity of monocytes. However, the lytic function of unseparated PBM cells was severely diminished by MHM23 monoclonal antibody. These results indicated that the cytotoxic cells were not T lymphocytes or NK cells. Also, neither lipopolysaccharide, nor purified recombinant glycosylated human IFN-γ augmented the cytotoxicity of PBM cells (confirming previous results[21]) or of monocytes (Table 1 and Fig. 2). However, the same preparation of IFN-γ enhanced the expression of major histocompatibility complex antigens on human monocytes (ref. 22 and J. Farrant, personal communication). It is noteworthy that although the dose and batch of lipopolysaccharide we used did not induce any significant monocyte cytotoxicity after a 24-h exposure (8% specific ^{51}Cr release), a very significant enhancement of cytotoxic activity of human monocytes was measured after a 48-h incubation period (57% specific ^{51}Cr release). Cytolytic activity was greatly reduced after monocytes were treated with anti-Leu-M3 (complement nonfixing, first layer) and rabbit anti-mouse Ig polyclonal antibody (second layer) plus rabbit complement (Fig. 3). This treatment lysed 70% of viable monocytes and depleted the cytolytic activity to a similar extent. As previously reported, the cytolytic effect of monocytes against T24 cells did not appear to be mediated directly by a cytotoxic soluble factor[17] or to require DNA synthesis for its induction (Fig. 4). Furthermore, the possibility that an IL-2-induced, PBM cell-secreted factor enhanced the cytotoxicity of monocytes seems unlikely, since the supernatant from PBM cells pulsed with IL-2 had no effect on monocyte cytotoxicity (data not shown). On the basis of these findings, we conclude that monocyte function can be directly influenced by IL-2. Moreover, our data are compatible with a report[23] showing that normal human monocytes express receptors for IL-2 on their surface. These observations, together with a recent study[24], show that IL-2 is not exclusively a lymphocytotrophic hormone. The major physiological role of IL-2 was thought to be activation of resting T lymphocytes (in G_1) to progress through the proliferative phases (S, G_2 and M) of the cell cycle. The biological significance of the effect of IL-2 on monocytes has yet to be determined, but our results imply that the IL-2 system may influence the behaviour of monocytes and their interactions with other cells during an immune response.

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Tumour necrosis factor and lymphotoxin genes map close to *H–2D* in the mouse major histocompatibility complex

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Tumour necrosis factor (TNF-α) and lymphotoxin (TNF-β) are related proteins, secreted by macrophages and lymphocytes respectively, which play a role in destruction of tumour cells and virally infected cells (for reviews see refs 1, 2). TNF-α is a non-glycosylated protein of relative molecular mass 17,000 (M_r 17 K), whereas TNF-β is a glycoprotein of M_r 25 K. Both TNF-α and TNF-β aggregate into multimers and act through the same receptor molecule on target cells. Genes encoding these two TNF proteins have been cloned from mouse and man[3–6] and in both are closely linked, being separated by ~1 kilobase (kb) of DNA[7,8]. In the mouse these genes are located on chromosome 17 (ref. 8), but in man they are on the short arm of chromosome 6 (ref. 9). This segment of chromosome 6 also contains the genes of the major histocompatibility complex (MHC), as does chromosome 17 in the mouse. To find out whether the TNF genes are located within the MHC, we used polymorphic restriction sites to analyse a panel of MHC congeneic and intra-MHC recombinant mouse strains. Initially, we mapped the TNF genes the *D* or *Qa* region in the distal half of the mouse MHC. We then studied a gene cluster encompassing part of the *D* and *Qa* regions and found the TNF genes are located 70 kb proximal to the *D* gene.

A portion of exon 4 of the TNF-α gene was used as a molecular probe to search for polymorphic restriction sites in different inbred mouse strains. Mouse strains AKR, A.SW, CAS3, C3H/HeJ, and C57BL/10 contain a *Bam*HI fragment of ~12 kb, and strains A/J, BALB/c and DBA/2 possess a smaller *Bam*HI fragment of ~10 kb. To confirm that the TNF genes are linked to the MHC, we analysed strains B10.A and B10.D2. The MHC in these mice is derived from strains A/J and DBA/2, respectively, but has been placed on the genetic background of

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Table 1 MHC alleles of inbred and recombinant mouse strains

Strain	MHC donor strains	Genetic locus							
		K	*I*	*S*	*D*	*Qa-2*	*Tla*	*Qa-1*	*Hmt*
A/J		k	k	d	d	a	a	a	a
AKR		k	k	k	k	b	b	b	a
A.SW		s	s	s	s	a	b	b	a
BALB/c		d	d	d	d	a	c	b	a
C3H/HeJ		k	k	k	k	b	b	b	a
C57BL/10		b	b	b	b	a	b	b	a
CAS3		c3	c3	c3	w3	a	c3	c3	b
DBA/2		d	d	d	d	a	c	b	a
B10.A	A/WySnSg	k	k	d	d	a	a	a	a
B10.D2	DBA/2	d	d	d	d	a	c	b	a
C3H.OH	DBA/2, C3H/J	d	d	d|	k	b	b	b	a
C3H.OL	DBA/2, C3H/J	d	d|	k	k	b	b	b	a
CAS3(R1)	CAS3, C3H/HeJ	c3	c3	c3	w3	a|	b	b	a
CAS3(R4)	C3H/HeJ, CAS3	k	k	k	k	b	b	b|	b
CAS3(R11)	C3H.SW, CAS3, C3H/HeJ	b|	c3	c3|	k	b	b	b	a

Alleles, based on serological identification, are listed according to refs 10 and 11. Locations of recorded crossovers are indicated by vertical lines.

C57BL/10. The smaller *Bam*HI fragment was found in both these congenic strains (Fig. 1*a*), confirming the linkage of the TNF genes to the MHC.

Next we analysed two congenic mouse strains containing a recombinant MHC derived from two haplotypes distinguishable for the TNF genetic marker. In mouse strains C3H.OH and C3H.OL, the proximal portion of the MHC (oriented towards the centromere) is of DBA/2 origin, whereas the distal portion (oriented towards the telomere) is of C3H origin (see Table 1 and ref. 10). The recombination occurred between *I* and *S* in strain C3H.OL, and between the *S* and *D* region marker loci in C3H.OH. Southern blot analysis of both strains using the TNF-α probe revealed the larger *Bam*HI fragment as in C3H mice and therefore located the TNF genes distal to the *S* region (Figs 1*a*, 2*a*).

We then analysed three intra-MHC recombinant mouse strains, CAS3(R1), CAS3(R4) and CAS3(R11), obtained from matings between C3H/HeJ and CAS3 mice[11]. We found a *Bgl*I fragment of ~23 kb with the TNF probe in CAS3 and a smaller fragment of ~19 kb in C3H/HeJ (Fig. 1*b*). Analysis of the three recombinant strains (Fig. 1*b*) revealed the larger fragment in CAS3(R1) and the smaller one in CAS3(R4) and CAS3(R11). Comparison of this result with the location of the breakpoints and the constellation of the recombinant haplotypes maps the TNF genes distal to the *S* and proximal to the *Tla* region marker loci, within the *D* or *Qa* region of the mouse MHC (Fig. 2*a*).

A 500-kb gene cluster with 13 class I genes, spanning the breakpoint between the *D* and *Qa* regions, has been cloned from the MHC of the BALB/c mouse[12] (Fig. 2*b*). To find out whether this cluster also contains the TNF genes, we analysed cosmid clones constituting the cloned region[12] by dot-blot hybridization. Only cosmid clone II 3.5, located at the proximal end of the cluster[12], gave a positive signal as strong as that obtained from the TNF-α gene used as a positive control (data not shown). This clone was digested with several restriction enzymes and analysed by Southern blot hybridization (Fig. 3*a*). The results, together with a comparison of the restriction maps of cosmid clone II 3.5 (ref. 12) and a mouse TNF genomic clone[8], located the TNF-α and -β genes to a region ~70 kb upstream of the *D* gene (Figs 2*b* and 3*b*). The TNF genes are transcribed in the opposite direction to the class I genes of this cluster.

The MHC, a large genetic region on mouse chromosome 17, encodes class I and class II cell-surface molecules that present foreign antigens to T lymphocytes (for reviews see refs 13, 14). Several class I and class II genes with unknown functions and 6 genes encoding complement components and steroid 21-hydroxylase have been identified in the mouse MHC (Fig. 2*b*). The location of the TNF genes at the proximal end of the *D*/*Qa* region gene cluster is close to genes in the MHC which are also structurally and functionally unrelated to class I and class II genes. It is curious that all these genes (C2/Bf; Slp/C4; 21-OHA/21-OHB; and TNF-α/TNF-β) are organized in pairs of related genes. It is not known whether the tight linkage between genes encoding molecules involved in the recognition (class I and class II) and effector (complement and TNF) phases of the immune response has any functional significance. TNF-α augments transcription of class I genes[15].

In man, certain diseases are associated with the presence of particular MHC alleles in affected individuals (for review see

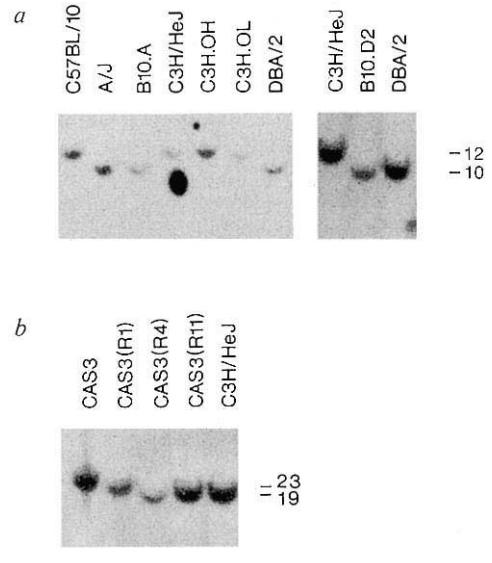

Fig. 1 Southern blot analysis of polymorphic restriction fragments containing the TNF-α gene. *Bam*HI (*a*) and *Bgl*I (*b*) were used to digest liver DNA from the mouse strains indicated. After agarose gel electrophoresis and transfer to Zeta probe membranes[20], hybridization was as described[21] using as a probe an oligolabelled[22] 450-bp *Hin*dII–*Eco*RI fragment derived from exon 4 of the TNF-α gene[8]. Sizes of the TNF-specific restriction fragments (in kb) were determined using restriction fragments of phage λ as markers.

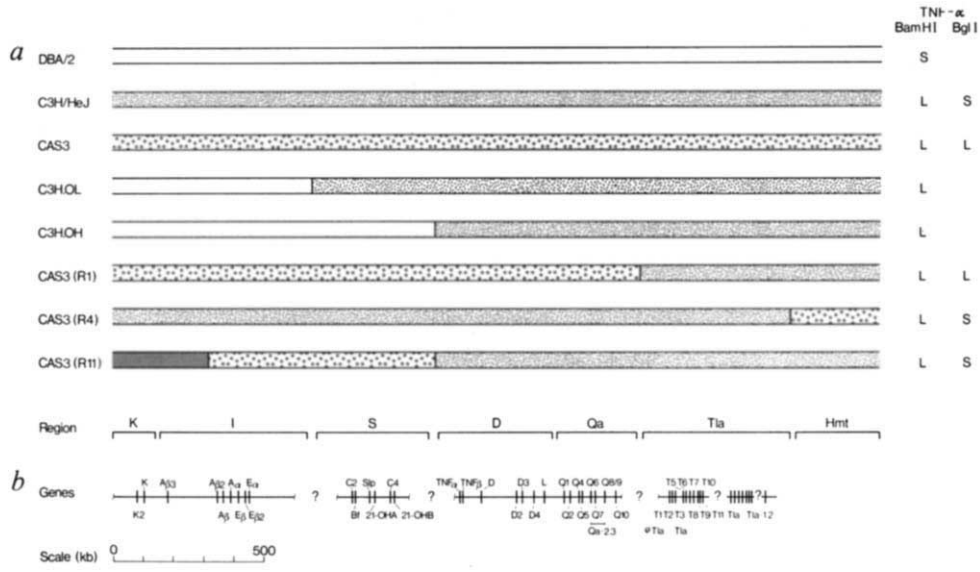

Fig. 2 Location of the TNF-α and -β genes in the mouse MHC. *a*, Polymorphic *Bam*HI and *Bgl*I fragments map the closely linked TNF-α and β-genes to the *D* or *Qa* region of the MHC. Different MHC halplotypes are distinguished by different textures to highlight the origin of MHC DNA in the recombinant mouse strains. Vertical lines, recombinational breakpoints; letters, regions of the MHC defined by them. *Hmt*, a gene which interacts with a maternally inherited factor to determine a cell-surface antigen (Mta), maps between the *Tla* and *Upg*-1 marker loci, about 2 cM distal to *D* (ref. 23) and is separated from *Tla* by the recombinational breakpoint in mouse strain CAS3(R4) (ref. 15). TNF-α alleles, as determined by Southern blot analysis using the restriction enzymes *Bam*HI and *Bgl*I (Fig. 1), are shown as S (small fragment) and L (large fragment). *b*, Gene clusters cloned from the MHC of the BALB/c mouse drawn to scale and identified genes are indicated by vertical lines (additional data from refs 12, 21, 24, 25). Class I genes map to the *K*, *D*, *Qa* and *Tla* regions, class II genes to the *I* region and the complement and 21-hydroxylase genes to the *S* region. ?, Unknown molecular distance between gene clusters. All class I genes of the *Tla* region are located between the recombinational breakpoints in CAS3(R1) and CAS3(R4) (M. Kiefer, K.F.L. and M.S., unpublished data).

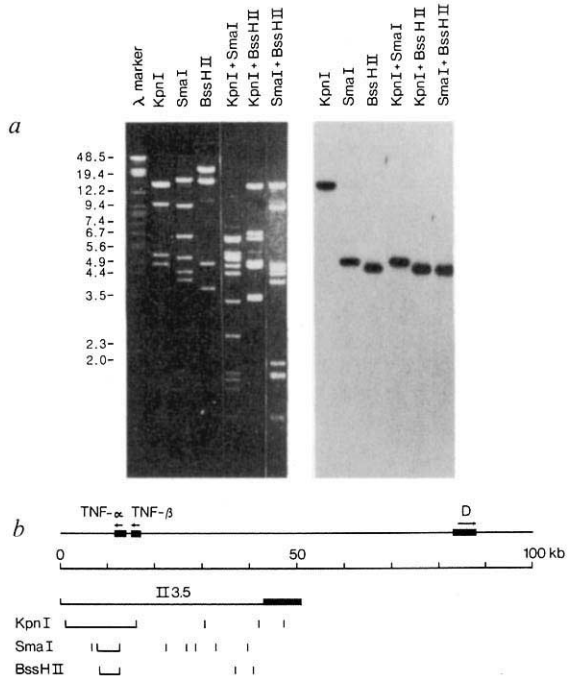

Fig. 3 Location of the TNF genes 70 kb proximal to the *D* gene in the BALB/c mouse. *a*, Fragments resulting from single and double digestions of cosmid clone II 3.5 using several enzymes (*Kpn*I, *Sma*I and *Bss*HII are shown) were separated by agarose gel electrophoresis (left panel), transferred to a Zeta probe membrane and hybridized with the TNF-α probe (right panel) as in Fig. 1. Sizes of phage λ marker framents are given in kb. *b*, Location and orientation of the TNF genes on cosmid clone II 3.5 based on hybridization analysis and restriction map comparison. *Kpn*I, *Sma*I and *Bss* HII restriction sites are shown for the region defined by clone II 3.5 only[12]. Solid box, cosmid vector portion of clone II 3.5; horizontal lines, restriction fragments hybridizing to the probe and therefore containing exon 4 of TNF-α. Orientation of transcription (5′→3′) is indicated by arrows. For a complete map of the 500-kb gene cluster see Fig. 2*b* and ref. 12.

ref. 16). From their location in the mouse MHC, the TNF genes are presumably closely linked to the HLA-B gene in man, a location consistent with recent mapping data[17]. Several rheumatic diseases are associated with the HLA-B27 allele, notably ankylosing spondylitis, an inflammatory disease of vertebral joints[16]. It is still unknown whether ankylosing spondylitis is caused by the B27 allele itself or by an allele of a second gene in linkage disequilibrium with B27. TNF-α stimulates collagenase production by human synovial cells[18] and resorption of proteoglycan in cartilage[19]. Thus an abnormal TNF gene could indeed be involved in ankylosing spondylitis and other inflammatory diseases.

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