Volume 14 Number 19 1986 Nucleic Acids Research

The genes for tumor necrosis factor (TNF-alpha) and lymphotoxin (TNF-beta) are tandemly arranged on chromosome 17 of the mouse

S.A.Nedospasov[1,2], B.Hirt[1], A.N.Shakhov[2], V.N.Dobrynin[3], E.Kawashima[4], R.S.Accolla[5] and C.V.Jongeneel[5]*

[1]Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland, [2]Institute of Molecular Biology, and [3]Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR, [4]Biogen S.A., Route des Acacias 46, CH-1227 Carouge/Geneva and [5]Ludwig Institute for Cancer Research, Lausanne Branch, CH-1066 Epalinges, Switzerland

Received 31 July 1986; Accepted 9 September 1986

ABSTRACT

We have isolated clones containing the gene for tumor necrosis factor (TNF-alpha) from a mouse genomic library. Four out of five clones containing the TNF-alpha gene also hybridized to a human lymphotoxin (TNF-beta) probe. We constructed a restriction enzyme cleavage map of a 6.4 kb region from one of the genomic clones. From partial sequencing data and hybridizations with exon-specific oligonucleotide probes, we conclude that this region contains the mouse TNF-alpha and TNF-beta genes in a tandem arrangement, that they are separated by only about 1100 bases, and that their intron-exon structure is very similar to that seen in man. We probed genomic blots of DNA from human/mouse hybrids containing single mouse chromosomes for the presence of the mouse TNF genes. The results show that the genes are located on mouse chromosome 17, which also contains the major histocompatibility complex. Therefore, both the mouse and the human TNF genes are tandemly arranged and located on the same chromosome as the MHC.

INTRODUCTION

Mononuclear cells of the reticuloendothelial system secrete, after stimulation, cytokines that have a necrotic effect on tumors, and are cytotoxic to many types of cultured transformed cells (1-6). An often confusing mass of data has been recently clarified by the demonstration (7, 8) that most if not all cytotoxic activities produced by mononuclear cells can be attributed to the products of only two genes, which code for tumor necrosis factor (hereafter designated as TNF-alpha) and for lymphotoxin (TNF-beta).

TNF-alpha has been shown to be identical to cachectin (9), the protein responsible for the wasting response associated with some bacterial and parasitic infections (10), and to the monocyte-derived cytotoxin (7). The cachectin activity of TNF-alpha seems to be due to a specific inhibition of the expression of lipoprotein lipase in adipocytes (11, 12). In contrast to its cytotoxic activities on tumor cell lines, TNF-alpha behaves like a cytokine for many types of normal cells: it induces the expression of type I histocompatibility antigens and of other surface markers on normal endothelial

cells (13, 14), enhances the growth of fibroblasts (15), and stimulates the production of collagenase and prostaglandin E2 (16). The latter effect may reflect TNF-alpha's ability to stimulate the release of IL-1 by endothelial cells (17). TNF-alpha seems to be produced mostly, if not exclusively, by adherent cells of the monocyte / macrophage lineage (2, 7, 8).

TNF-beta, or lymphotoxin, is produced by T lymphocytes (18, 19). While it was originally detected as a secreted protein in the culture medium of stimulated lymphocytes, there is evidence for its specific delivery to target cells by cytotoxic T-cells and its involvement in the subsequent degradation of the target cell DNA (20, 21).

There are many similarities in the induction and the mode of action of the two TNFs. In populations of peripheral blood mononuclear cells, their synthesis is induced by bacterial endotoxins, but also by mitogens, by phorbol esters, and by the T-cell growth factor, interleukin-2 (2, 8, 22). The cytotoxic effects that they exhibit toward tumor cell lines are quite similar (7, 23). Their cytotoxicity is synergistic with that of immune interferon, another cytokine, and it has been postulated that the in vivo effects of TNF-alpha/-beta and immune interferon are dependent upon each other (22, 24, 25). This hypothesis has been strengthened by the recent demonstration that immune interferon can enhance the expression of TNF receptors in several tumor lines (26, 27).

Because of the obvious potential of these molecules in clinical applications, great efforts have been made to characterize their genes and to have them expressed to high levels in prokaryotic hosts. These efforts have brought about the cloning and sequencing of the genes and cDNAs of human TNF-alpha and TNF-beta (28-32), of the cDNA of mouse TNF-alpha (33,34), and of the gene and cDNA of rabbit TNF-alpha (35,36). As a result, there are now abundant sources of pure recombinant TNF-alpha and TNF-beta. The sequencing of both proteins from human sources revealed a significant structural homology, as the secreted forms share 35% of their amino acid sequence (37,38). The homology also extends, albeit to a lesser degree, to the DNA region upstream of the two genes (31), suggesting similarities in the way their expression is regulated. Cytogenetic studies have shown that the genes for human TNF-alpha and TNF-beta are located on the short arm of chromosome 6, in close proximity to the major histocompatibility complex (31). An independent analysis of a lambda clone containing the TNF-alpha gene has established that the genes for TNF-alpha and TNF-beta are tandemly arranged in the human genome (32, and CSHSQB, in press).

In the present paper, we show that the genes for TNF-alpha and TNF-beta are adjacent to each other in the mouse genome, and that their configuration and nucleotide sequence are highly conserved between mice and humans. Moreover, they are located on chromosome 17, which also contains the mouse major histocompatibility complex.

MATERIALS AND METHODS

Isolation of the TNF genes from a mouse genomic library

The mouse genomic library we used was obtained from Drs L. Mori and M. Steinmetz. It had been derived from the LH8 T-cell lymphoma, which was isolated from Radiation Leukemia Virus-infected C57Bl/6 mice and has T suppressor cell characteristics (39). A partial Mbo I digest of LH8 cell DNA was cloned into the BamH I site of the EMBL3 lambda phage vector described by Frischauf et al. (40). 10^6 plaques were screened by hybridization with a synthetic oligonucleotide corresponding to nucleotides 554 to 568 in the human TNF-alpha cDNA sequence (28). 23 out of the 25 bases of this oligonucleotide are identical to the homologous mouse sequence. The probe was labeled by filling in overlapping complementary oligonucleotides with ^{32}P-labeled nucleotides using the Klenow fragment of E. coli DNA polymerase I. Filters were washed in 4xSSC at 60°C. Positive plaques were replated and screened until a homogeneous population was obtained, and recombinant lambda phage DNA was prepared using standard techniques.

The exon-specific mTNF-alpha oligonucleotides that we used to determine the relative positions of exons and restriction sites were located as follows on the cDNA sequence (33): exon 1, nt 223-272; exon 2, nt 350-369; exon 3, nt 399-418; exon 4, nt 852-870.

Subcloning and restriction mapping

Selected fragments were isolated from EcoR I digests of the lambda clones by electrophoresis in low temperature gelling agarose (41), and were subcloned into the mWB238 or mWB239 filamentous phage vectors (42, 43). All sequencing was done using the dideoxynucleotide chain terminator method of Sanger (44). Maps for restriction endonuclease cleavage sites were established by standard strategies, and verified by hybridizing selected subclones to Southern blots. These routine hybridizations were performed in 6xSSC at 68°C, using 0.25% non-fat dry milk as a blocking agent (45).

Detection of TNF genes in mouse/human hybrid cells

The derivation and cytogenetic analysis of hybrid cells that selectively lose mouse chromosomes has been described in detail elsewhere (46, 47). In

order to detect TNF genes in these hybrids, whole DNA (5 ug) was digested with 15 units of the appropriate restriction endonuclease, separated by electrophoresis in agarose gels, blotted onto nylon membranes (Amersham Hybond N), and linked to the membrane by UV irradiation. Hybridizations were performed according to Church and Gilbert (48), using ^{32}P-labeled RNA probes synthesized in vitro with the SP6 phage RNA polymerase (49). As templates for the polymerase, we used the following fragments cloned in the pSP6 vectors: (i) TNF-alpha: a 450 bp Hinc II to EcoR I fragment derived from exon 4; (ii) TNF-beta: a 950 bp Pvu II fragment extending from the first intron to the middle of exon 4; (iii) H-2K^b: a Sma I fragment covering the second exon of the H-2K^b gene (coding for the alpha-1 domain; Weiss et al., 1983).

RESULTS

Analysis of genomic clones

We screened a genomic library derived from the LH8 mouse T-cell lymphoma line (39) with an oligonucleotide probe derived from the sequence of exon 4 of the human TNF-alpha (hTNF-alpha) (28). This oligonucleotide matches the homologous mouse sequence (33) in 23 out of 25 positions. We identified and plaque-purified five genomic clones that hybridized to the oligonucleotide. To verify that they actually contain the TNF-alpha gene, we probed each clone with four different oligonucleotides whose sequence matches parts of the four presumed exons of the mouse TNF-alpha (mTNF-alpha) cDNA. All five clones contain a 2.8 kb EcoR I fragment that hybridizes to all four oligonucleotides. Therefore, most of the mTNF-alpha gene is contained in this EcoR I fragment.

Four out of the five clones that contain the mTNF-alpha gene also hybridized with a genomic probe derived from exon 4 of the human TNF-beta gene (EcoR I to Pst I; see ref. 31). These clones all shared a 1.9 kb BamH I fragment hybridizing to the hTNF-beta probe, and three out of four contained a 3.6 kb EcoR I fragment hybridizing to the same probe.

Assuming that the human TNF-beta probe is actually specific for the homologous mouse gene, these results indicate that the mouse TNF-alpha and TNF-beta genes are closely linked (the genomic clones contain inserts ranging from 10 to 25 kb). In order to verify this hypothesis, we decided to analyze further one of the clones, which contains 2.8 kb and 3.6 kb EcoR I fragments that hybridize to TNF-alpha and TNF-beta probes respectively.

Structure of the mTNF-alpha gene

The 2.8 kb EcoR I fragment which contains most of the mTNF-alpha gene by the criteria mentioned above was subcloned into the mWB238 filamentous phage

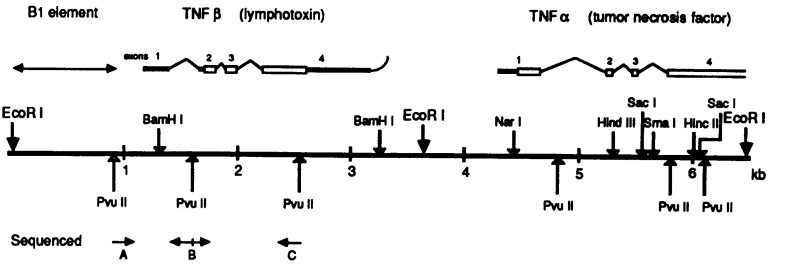

Figure 1
Physical map of the mouse TNF gene cluster. The map covers the two EcoR I fragments that we subcloned and mapped in detail. The positions and structures of the mRNAs (top) were deduced from a combination of restriction mapping, exon-specific hybridizations, sequencing, and homology to the human genes. Thick lines indicate untranslated regions, open boxes correspond to coding regions. They could still be inaccurate in minor details. The arrows at the bottom indicate the direction and extent of sequencing, with regions A, B and C corresponding to the panels in Figure 2.

vector (42, 43). Since the mTNF-alpha cDNA sequence contains a unique EcoR I site (33), we assumed that this would define one of the ends of the clone. We mapped the position relative to this EcoR I site of other restriction endonuclease cleavage sites (for the Hinc II, PvuII, Nar I and Sac I enzymes) predicted from the cDNA sequence. All the sites were found at positions consistent with the map shown in Figure 1. A 450 bp fragment extending from the Hinc II site to the EcoR I site was subcloned and partially sequenced to confirm that we were dealing with the mTNF-alpha gene. Our sequence is in complete agreement with the published cDNA sequence (33).

Further restriction sites (for Hind III, Sma I, Sac I, and Pvu II) are located in regions presumed to be introns by analogy to the human gene. The positions of these sites relative to the presumed exons were determined by probing relevant digests with mTNF-alpha exon-specific oligonucleotides. This analysis confirmed the relative positions of exons and restriction sites shown in Figure 1, implying that the intron-exon distribution of the mouse gene is very similar to that of the human gene. The mTNF-alpha gene structure shown in Figure 1 was inferred from the cDNA sequence, our restriction mapping data, and the known structure of the human gene. Therefore, minor errors as to the exact sizes and positions of introns and exons could still be present.

Structure of the mTNF-beta gene

The 3.6 kb EcoR I fragment that hybridizes to the hTNF-beta probe was subcloned into the mWB238 vector. This fragment contains the 1.9 kb TNF-beta-

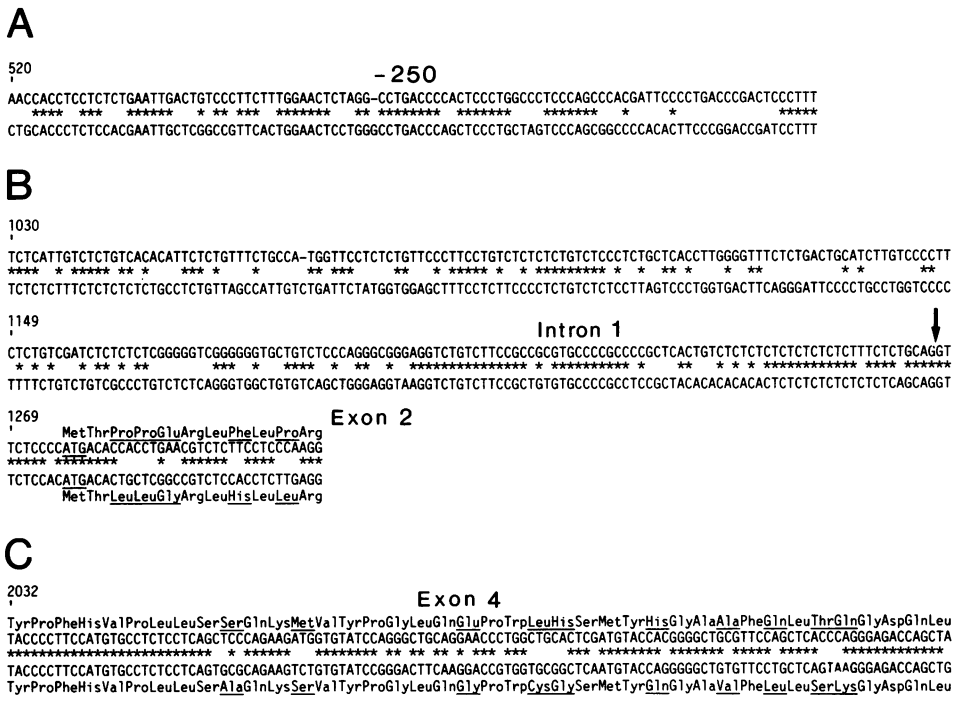

Figure 2
Comparison between the nucleotide sequences of the human and mouse TNF-beta genes. The sequences of the human gene (top) and the mouse gene (bottom) were aligned to maximize homology, and occasional gaps (hyphens) were inserted to maintain this optimal alignment. Asterisks mark the positions where the nucleotides are identical in both sequences. The nucleotide numbering of the human TNF-beta gene is according to Nedwin et al. (31). The boundary between intron 1 and exon 2 is marked with an arrow, and the translation of the sequences is indicated in the areas known to be part of the coding sequence of the human gene. Within translated regions, amino acids that differ between the mouse and human gene are underlined. A: region between -300 and -200 relative to the 5' end of the mRNA; B: part of the first intron and beginning of the second exon, including part of the signal peptide; C: part of the fourth exon, in the coding sequence for the secreted protein. These regions correspond to the arrows in Figure 1.

specific BamH I fragment mentioned before, in the configuration shown in Figure 1. We also mapped the cleavage sites for Pvu II (see Figure 1), and subcloned the two internal Pvu II fragments into the mWB239 vector. Partial sequencing of these cloned Pvu II fragments (arrows in Figure 1) and comparison of the sequences to the human TNF-beta gene by the matrix method of Pustell and Kafatos (51) revealed stretches of homology. The distribution of

these homologies allowed a line-up of our restriction map of the mouse gene with the known sequence of the human gene (31). Within the accuracy of fragment length measurements in agarose gels, the distances between homologous regions are the same in the two species.

Figure 2 shows a comparison of the stretches of mouse sequence that we obtained with their human homologs. Several features are worth noting: (i) Detectable homology between the human and mouse sequences extends at least 300 nucleotides upstream of the mRNA cap site (panel A), even though the level is rather low (55%) and homologies are unevenly distributed. (ii) The second region that we have sequenced (panel B) covers most of the first intron and the beginning of the second exon. Within this region, the homology becomes stronger as one gets closer to the intron/exon boundary. Curiously, stretches of alternating nucleotides in the intron (...TCTCTCTC...) are among the conserved features. The splice acceptor is entirely conserved, as well as the first amino acid of the signal peptide (assuming that the initiator Met is not conserved). (iii) Within the coding sequence for the secreted portion of the protein (panel C), conservation is very high (75% homology at the amino acid level in the short stretch we sequenced). This figure seems lower in the signal peptide (5 out of the 11 amino acids for which we have information are different).

Hybridization of digests of the mTNF-beta gene to nick-translated total mouse DNA reveals the presence of a highly repeated element at the left end of our map, upstream of the first Pvu II site. This was confirmed by hybridizing probes derived from this region to Southern blots of total mouse DNA. An 8-kb fragment derived from the 3'-terminal region of the mouse elastase II gene and containing two B1 elements (a kind gift of B. Stevenson) hybridized to the same Pvu II and BamH I fragments as nick-translated total mouse DNA. This strongly suggests that there is a B1 element (19) upstream of the TNF-beta gene. The position of the B1 element is homologous to that of an Alu sequence found in the human TNF locus (Nedospasov et al., CSHSQB, in press).

Linkage between the TNF-alpha and TNF-beta genes

The first indication that the genes are adjacent to each other came from data showing that a probe derived from the mTNF-alpha 2.8 kb EcoR I fragment, in contrast to a smaller probe from the 3' terminal Hinc II - EcoR I fragment, detects a Hind III fragment and a Sma I fragment that also hybridize to a mTNF-beta probe. The simplest possible explanation was that the EcoR I site at the left end of TNF-alpha is also the site defining the right end of TNF-beta. This hypothesis was verified by hybridizing TNF-alpha and TNF-beta

Table I: Characteristics of human/mouse hybrid cells

Hybrid clone	Mouse chromosomes[a]	H-2K expression[b]
A2.KD clone 1	17 (76%)	74%
H4	16 (89%)	1%

[a] Chromosomes detected by karyotype analysis, and percentage of metaphases examined containing the chromosome. No other mouse chromosomes could be detected.

[b] Percentage of cells expressing the marker as detected by staining with an H-2K specific antibody and FACS analysis.

probes to DNA from the original lambda clone digested with combinations of Pvu II, BamH I, Hind III, Sma I, and EcoR I and blotted onto filters. All of the results were consistent with the map presented in Figure 1. Therefore, our data show that the mouse genes for TNF-alpha and TNF-beta are extremely close to each other. Assuming that the two genes have equivalent structures in mice and men (which is likely from our other data), the polyadenylation site of mTNF-beta is only 1.1 kb away from the cap site of the mTNF-alpha mRNA (32, and Nedospasov et al., CSHSQB, in press).

The mouse TNF genes are located on chromosome 17

In order to establish the chromosome localization of the TNF cluster, we performed hybridizations of probes derived from the mTNF-alpha and mTNF-beta genes to blots of restriction endonuclease-digested DNA extracted from human, mouse, and human-mouse hybrid cells. The hybrid cells were derived from fusions of a human lymphoblastoid cell line, RJ 2.2.5, with unstimulated spleen cells from Balb/c mice (46, 47). Hybrids produced in this fashion selectively lose mouse chromosomes rather than human ones, and can be used to map mouse genes. The characteristics of the human-mouse hybrids that we used are summarized in Table I.

Figure 3 shows the results obtained when a Southern blot of total EcoR I-digested DNA from the RJ 2.2.5 cells, the hybrids, and control mouse samples was probed with a mTNF-beta probe. In agreement with the data of Nedwin et al. (31), we find that the human TNF-beta gene is located on a 2.4 kb fragment, while the mouse gene is on a 3.6 kb fragment. The 3.6 kb mouse-specific band is present only in the AD2.KD hybrid, which contains the mouse chromosome 17, and was selected for expression of mouse H-2K surface antigens. No mouse chromosomes other than 17 could be detected in over 40 metaphases of the AD2.KD hybrid. We confirmed the segregation of the TNF genes with

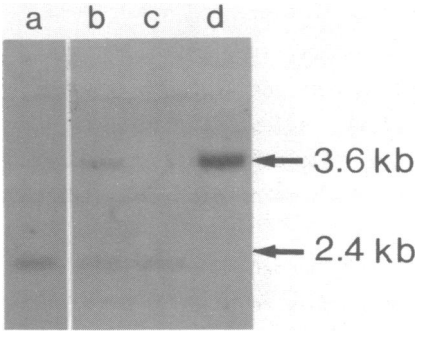

Figure 3.
Hybridization of a mTNF-beta-specific probe to EcoR I digested DNAs. Lane a: RJ 2.2.5, the human parent of the hybrids; lane b: A2.KD clone 1 hybrid line (mouse chromosome 17); lane c: H4 hybrid line (mouse chromosome 16); lane d: M12, mouse B cell line of Balb/c origin. See Table I for a description of the hybrids.

chromosome 17 by probing the same gel with a ^{32}P-labeled RNA specific for the mouse H-2K locus: H-2K -specific bands were seen only in the AD2.KD hybrid (data not shown). Hybridization of the mTNF-beta probe to a blot of BamH I-digested DNAs revealed the expected 2.0 kb mouse-specific fragment, while the human gene is located on a 20 kb fragment. In agreement with the results derived from EcoR I-digested DNAs, the 1.9 kb mouse-specific BamH I fragment is present only in the hybrid containing chromosome 17 (data not shown). We also probed blots of mouse-human hybrid DNAs with a mTNF-alpha clone, and obtained similar results (not shown).

These results establish that the mouse TNF genes are located on chromosome 17, which also contains the major histocompatibility complex genes. The exact location of the TNF genes relative to the MHC is currently under investigation.

DISCUSSION

The data presented in this paper show that the structure of the mouse genes for TNF-alpha (tumor necrosis factor, cachectin) and TNF-beta (lymphotoxin) closely resembles that of their human counterparts (31, 32, and Nedospasov et al., CSHSQB, in press). The tandem configuration of the genes, as well as their intron/exon distribution, are identical within the limits of our measurements. In addition, both genomes contain a SINE repetitive element in similar positions (52, and Nedospasov et al., CSHSQB, in press). At the

nucleotide sequence level, the mouse and human genes for TNF-beta are highly homologous, with the highest degree of homology found within the portion coding for the secreted form of the protein, as expected from the conservation of function and the lack of species specificity of TNF. Sequences upstream from the mRNA cap site are less conserved. Like the human genes, the mouse TNF genes are located on the same chromosome as the major histocompatibility complex.

The close proximity of the genes for TNF-alpha and TNF-beta reinforces the notion that they are closely related, and most likely evolved from a gene duplication event followed by insertion of the exons coding for the signal peptide (which is very different between the two genes) and for the 5' untranslated sequence. It is worth noting that even though the level of sequence homology indicates that the two proteins may have diverged as long as several hundred million years ago (31), they have remained very closely linked. Previous reports (23, 31, 38) have shown that the homologies between the two proteins are concentrated in exon 4, which codes for 80% of the sequence of the secreted form in both genes. This structural homology is mirrored by functional homologies, since the two proteins share the same receptor and have very similar effects on their target cells (7, 23).

The cDNA sequences of the two TNF genes clearly indicate that they are transcribed as non-overlapping mRNAs. In addition, each gene seems to have its own promoter and polyadenylation signals (31). The two genes are expressed in distinct cell populations, and are induced with quite different kinetics (8). It would be unusual for two genes in such close physical proximity to be regulated in a completely independent fashion. Therefore, we believe that a thorough study of the regulation of the TNF genes will provide interesting insights into the more general problem of tissue-specific expression of related genes. It has already been noted that there is detectable homology between the upstream, presumably regulatory regions of TNF-alpha and TNF-beta. This may reflect similarities in the signals used to turn the genes on, but leaves no clues as to why they are expressed in different cells. Since expression of the TNF genes is controlled at least in part at the transcriptional level (53), one might expect the determinants of tissue specificity to be among the non-conserved features of the controlling regions of the two genes.

The linkage of the TNF genes to the MHC is conserved between mice and men, at least at the level of chromosomal localization. We have no evidence at the present time to tie this linkage to a regulatory coordination with

other genes of the MHC, but it is interesting at least as an example of functional clustering of genes, and gives TNF-alpha and TNF-beta a firmer place among both the effectors and the modulators of the immune response.

ACKNOWLEDGEMENTS

We thank L. Mori and M. Steinmetz (Basel Institute for Immunology) for the mouse genomic library. S.A.N. was partially supported by a fellowship from the Swiss Cancer League during the course of this work. The work was funded in part by grants from the Swiss National Science Fund.

*To whom correspondence should be addressed

REFERENCES

1. Granger,G.A. and Williams,T.W. (1968) Nature **218**, 1253-1254.
2. Carswell,E.A., Old,L.J., Kassel,R.L., Green,S., Fiore,N. and Williamson,B. (1975) Proc. Natl. Acad. Sci. USA **72**, 3666-3570.
3. Wright,S.A.C. and Bonavida,B. (1981) J. Immunol. **126**, 1279-1283.
4. Ruddle,N.H., Powell,M.B. and Conta,B.S. (1983) Lymphokine Res. 2, 23-31.
5. Old,L.J. (1985) Science **230**, 630-632.
6. Sugarman,B.J., Aggarwal,B.B., Hass,P.E., Figari,I.S., Palladino,M.A. and Shepard,H.M. (1985) Science **230**, 943-945.
7. Chroboczek Kelker,H., Oppenheim,J.D., Stone-Wolff,D., Henriksen-deStefano,D., Aggarwal,B.B., Stevenson,H.C. and Vilcek,J. (1985) Int. J. Cancer **36**, 69-73.
8. Nedwin,G.E., Sverdesky,L.P., Bringman,T.S., Palladino,M.A. and Goeddel,D.V. (1985) J. Immunol. **135**, 2492-2496.
9. Beutler,B., Greenwald,D., Hulmes,J.D., Chang,M., Pan,Y.C.E., Mathison,J., Ulevitch,R. and Cerami,A. (1985) Nature **316**, 552-554.
10. Beutler,B. and Cerami,A. (1986) Nature **320**, 584-588.
11. Beutler,B., Mahoney,J., Le Trang,N., Pekala,P. and Cerami,A. (1985) J. Exp. Med. **161**, 984-995.
12. Torti,F.M., Dieckmann,B., Beutler,B., Cerami,A. and Ringold,G.M. (1985) Science **229**, 867-869.
13. Collins,T., Lapierre,L.A., Fiers,W., Strominger,J.L. and Pober,J.S. (1986) Proc. Natl. Acad. Sci. USA **83**, 446-450.
14. Pober,J.S., Bevilacqua,M.P., Mendrick,D.L., Lapierre,L.A., Fiers,W. and Gimbrone,M.A. (1986) J. Immunol. **136**, 1680-1687.
15. Vilcek,J., Palombella,V.J., Henriksen-DeStefano,D., Swenson,C., Feinman,R., Hirai,M. and Tsujimoto,M. (1986) J. Exp. Med. **163**, 632-643.
16. Dayer,J.M., Beutler,B. and Cerami,A. (1985) J. Exp. Med. **162**, 2163-2168.
17. Nawroth,P.P., Bank,I., Handley,D., Cassimeris,J., Chess,L. and Stern,D. (1986) J. Exp. Med. **163**, 1363-1375.
18. Shacks,S.J., Chiller,J. and Granger,G.A. (1973) Cell. Immunol. 7, 313-321.
19. Leopardi,E. and Rosenau,W. (1984) Cell. Immunol. **83**, 73-82.
20. Schmid,D.S., Tite,J.P. and Ruddle,N.H. (1986) Proc. Natl. Acad. Sci. USA **83**, 1881-1885.
21. Duke,R.C., Chervenak,R. and Cohen,J.J. (1983) Proc. Natl. Acad. Sci. USA **80**, 6361-6365.
22. Sverdesky,L.P., Nedwin,G.E., Goeddel,D.V. and Palladino,M.A. (1985) J. Immunol. **134**, 1604-1608.

23. Gray,P.W., Aggarwal,B.B., Benton,C.V., Bringman,T.S., Henzel,W.J., Jarrett,J.A., Leung,D.W., Moffat,B., Ng,P., Sverdesky,L.P., Palladino,M.A. and Nedwin,G. (1984) Nature **312**, 721-724.
24. Lee,S.H., Aggarwal,B.B., Rinderknecht,E., Assisi,F. and Chiu,H. (1984) J. Immunol. **133**, 1083-1086.
25. Stone-Wolff,D.S., Yip,Y.K., Chroboczek Kelker,H., Le,J., Henriksen-deStefano,D., Rubin,B.Y., Rinderknecht,E., Aggarwal,B.B. and Vilcek,J. (1984) J. Exp. Med. **159**, 828-843.
26. Ruggiero,V., Tavernier,J., Fiers,W. and Baglioni,C. (1986) J. Immunol. **136**, 2445-2450.
27. Tsujimoto,M., Yip,Y.K. and Vilcek,J. (1986) J. Immunol. **136**, 2441-2444.
28. Pennica,D., Nedwin,G.E., Hayflick,J.S., Seeburg,P.H., Derynck,R., Palladino,M.A., Kohr,W.J., Aggarwal,B.B. and Goeddel,D.V. (1984) Nature **312**, 724-729.
29. Shirai,T., Yamagushi,H., Ito,H., Todd,C.W. and Wallace,R.B. (1985) Nature **313**, 803-806.
30. Marmenout,A., Fransen,L., Tavernier,J., Van der Heyden,J., Tizard,R., Kawashima,E., Shaw,A., Johnson,M.J., Semon,D., Muller,R., Ruysschaert,M.R., Van Vliet,A. and Fiers,W. (1985) Eur. J. Biochem. **152**, 515-522.
31. Nedwin,G.E., Naylor,S., Sakaguchi,A.Y., Smith,D., Jarrett-Nedwin,J., Pennica,D., Goeddel,D.V. and Gray,P.W. (1985) Nucl. Acids Research **13**, 6361-6373.
32. Nedospasov,S.A., Shakov,A.N., Turetskaya,R.L., Mett,V.A., Georgiev,G.P., Dobrynin,V.N. and Korobko,V.G. (1985) Dokl. Acad. Nauk SSSR **285**, 1487-1490.
33. Fransen,L., Muller,R., Marmenout,A., Tavernier,J., Van der Heyden,J., Kawashima,E., Chollet,A., Tizard,R., Van Heuverswyn,H., Van Vliet,A., Ruysschaert,M.R. and Fiers,W. (1985) Nucl. Acids Research **13**, 4417-4429.
34. Pennica,D., Hayflick,J.S., Bringman,T.S., Palladino,M.A. and Goeddel,D.V. (1985) Proc. Natl. Acad. Sci. USA **82**, 6060-6064.
35. Ito,H., Yamamoto,S., Kuroda,S., Sakamoto,H., Kajihara,J., Kiyota,T., Hayashi,H., Kato,M. and Seko,M. (1986) DNA **5**, 149-156.
36. Ito,H., Shirai,T., Yamamoto,S., Akira,M., Kawahara,S., Todd,C.W. and Wallace,R.B. (1986) DNA **5**, 157-165.
37. Aggarwal,B.B., Kohr,W.J., Hass,P.E., Moffat,B., Spencer,S.A., Henzel,W.J., Bringman,T.S., Nedwin,G.E., Goeddel,D.V. and Harkins,R.N. (1985) J. Biol. Chem. **260**, 2345-2354.
38. Aggarwal,B.B., Henzel,W.J., Moffat,B., Kohr,W.J. and Harkins,R.N. (1985) J. Biol. Chem. **260**, 2334-2344.
39. Mori,L., Lecoq,A.F., Robbiati,F., Barbanti,E., Righi,M., Sinigaglia,F., Clementi,F. and Ricciardi-Castagnoli,P. (1985) EMBO J. **4**, 2025-2030.
40. Frischauf,A.M., Lehrach,H., Poustka,A. and Murray,N. (1983) J. Mol. Biol. **170**, 827-842.
41. McMaster,G.K., Beard,P., Engers,H.D. and Hirt,B. (1981) J. Virol. **38**, 317-326.
42. Barnes,W.M., Bevan,M. and Son,P.H. (1983) Meth. Enzymol. **101**, 98-122.
43. Sahli,R., McMaster,G.K. and Hirt,B. (1985) Nucl. Acids Res. **13**, 3617-3633.
44. Sanger,F., Nicklen,S. and Coulson,A.R. (1977) Proc. Natl. Acad. Sci. USA **74**, 5463-5467.
45. Johnson,D.A., Gautsch,J.W., Sportsman,J.R. and Elder,J.H. (1984) Gene Anal. Techn. 1, 3-8.
46. Accolla,R.S., Scarpellino,L., Carra,G. and Guardiola,J. (1985) J. Exp. Med. **162**, 1117-1133.
47. Accolla,R.S., Jotterand-Bellomo,M., Scarpellino,L., Maffei,A., Carra,G. and Guardiola,J. (1986) J. Exp. Med. **164**, 369-374.

48. Church,G.M. and Gilbert,W. (1984) Proc. Natl. Acad. Sci. USA **81**, 1991-1995.
49. Butler,E.T. and Chamberlin,M. (1982) J. Biol. Chem. **257**, 5772-5778.
50. Weiss,E., Golden,L., Zakut,R., Mellor,A., Fahrner,K., Kvist,S. and Flavell,R.A. (1983) EMBO J. 2, 453-462.
51. Pustell,J. and Kafatos,F.C. (1984) Nucl. Acids Res. 12, 643-655.
52. Georgiev,G.P., Ilyin,Y.V., Chmeliauskaite,V.G., Ryskov,A.P., Kramerov,D.A., Skryabin,K.G., Krayev,A.S., Lukanidin,E.M. and Grigoryan,M.S. (1981) Cold Spring Harbor Symp. Quant. Biol. **45**, 641-654.
53. Beutler,B., Krochin,N., Milsark,I.W., Luedke,C. and Cerami,A. (1986) Science **232**, 977-980.