Hum Genet (1991) 87:231–233

Human Genetics

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# The gene for the type 1 tumor necrosis factor receptor (TNF-R1) is localized on band 12p13

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Received December 5, 1990

**Summary.** The gene coding for the type I (p55) tumor necrosis factor receptor (TNF-R1) has been localized on human chromosome 12, band 12p13.2, by in situ hybridisation using a biotinylated genomic probe.

## Introduction

Tumor necrosis factors alpha and beta (TNFA, TNFB) are structurally related cytokines with pleiotronic biological activities involved in several aspects of the inflammatory response (Old 1989; Beutler and Cerami 1988). They initiate their effects on cell function by binding to common cell surface receptors (Aggarval et al. 1985). Two distinct molecular species of TNF receptors (TNF-Rs), both of which bind TNFA and TNFB, have been identified. These two receptors differ in size and are expressed differentially in different cell lines (Hohmann et al. 1989; Engelmann et al. 1990). Recently, the cDNAs for both receptors have been cloned and their nucleic acid sequence determined (Loetscher et al. 1990; Nophar et al. 1990; Schall et al. 1990; Smith et al. 1990). There is a marked similarity in structure between the extracellular domains of the two TNF-Rs. Both are composed of repetitive cysteine-rich domains, homologous to repetitive structures found in the NGF receptor and in certain other cell surface proteins. On the other hand, the structure of the intracellular domains appears to be unrelated. Southern blotting analysis of human genomic DNA, using the cDNAs of the two TNF-Rs as probes, indicated that each is encoded by a single gene. We report here the localization of the gene encoding one of the two receptors – the type I or p55 TNF-R – on human chromosome 12 by nonradioactive in situ hybridization.

## Materials and methods

### *Chromosome preparations*

High-resolution chromosome preparations were obtained from phytohemagglutinin-stimulated blood cultures of healthy males after methotrexate synchronization (Camargo and Cervenka 1980, 1982).

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### *Probe*

A human genomic library (prepared from PLC/PRF/5 cells in the charon 28 vector, Shaul et al. 1984) was screened under high stringency conditions with a clone corresponding to a 0.98 kb fragment of TNF-R1 cDNA (refered to as C2 in Nophar et al. 1990). The clones obtained were mapped using oligonucleotides (corresponding to nucleotides 40–60, 150–166, 458–478, 422–442, 1924–1958 of the type I TNF-R cDNA according to Nophar et al. 1990) and the 16 kb G3 clone was used as total phage DNA as a probe for in situ hybridization. The probe was labeled by nick-translation with bio-11-dUTP (Sigma, La Verpillière) according to the BRL protocol and then prepared according to Cherif et al. (1990).

### *Prehybridization*

Competitive hybridization was performed to eliminate hybridization of repetitive sequences contained within the 16 kb probe. In brief, nick-translated probe and competitor DNA (human placenta DNA, Sigma, sonicated to 300–500 base pairs) were repared at final concentrations of 1 μg/ml (probe) and 100 and 50 μg/ml (competitor human DNA) in a hybridization mixture as described by Cherif et al. (1990). After denaturation at 70°C for 5 min, DNA was preannealed by incubation of the hybridization mixture at 37°C for 1 h and 3 h (Kievits et al. 1990).

### *In situ hybridization*

In situ hybridization was carried out according to Cherif et al. (1990). The hybridization mixture (50 ml), corresponding to a final probe concentration of 1 μg/ml, was placed on the slide, covered with a coverslip, and sealed with rubber cement. After overnight incubation at 37°C, the slides were washed according to the protocol previously described.

### *Probe detection and R-banding*

The hybridization signal was revealed by avidin-conjugated fluorescein isothiocyanate (FITC; 5 mg/ml, Vector Laboratories, Burlingame) and amplified with additional layers of biotinylated goat anti-avidin antibody (5 mg/ml, Vector laboratories) according to Pinkel et al. (1986). R-bands were obtained by a modification of the technique of Camargo and Cervenka (1980, 1982) omitting the Giemsa staining step. After staining with Hoechst 33258, the slides were UV-irradiated (365 nm) for 20 min, and then immersed in Earle solution at 87°C (Bernheim and Berger 1981) for 2 min. A second round of signal amplification was carried out to ease the detection of hybridization signal, and the chromosomes were finally counterstained with propidium iodide (1 mg/ml) in an antifade solution (Johnson et al. 1981).

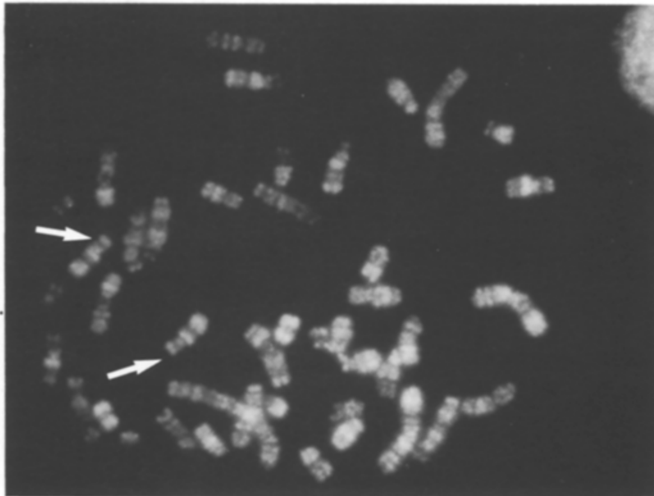

**Fig. 1.** R-banded metaphase. The two chromosome 12 homologues are indicated by *arrows*

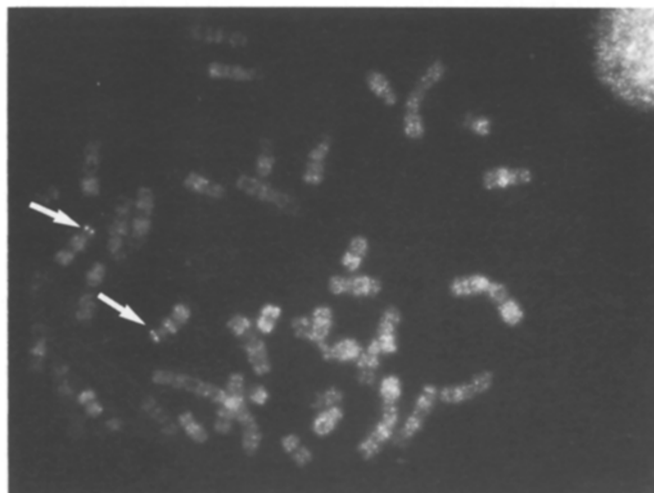

**Fig. 2.** In situ hybridization with the TNF-RI biotinylated probe. The double spots on chromosomes 12 are indicated by *arrows*

The slides were screened with a Zeiss fluorescence microscope equipped with filter combination No. 487709 for detection of the signal (FITC) and No. 487715 for chromosome identification. The hybridization signal appeared as yellow-green twin spots over sister chromatids on R-banded chromosomes stained in red. Microphotographs were taken with Kodak Ektachrome 400 film.

## Results

The localisation of the hybridized probe was unambiguous after analysing 30 metaphases. A spot on both chromatids (Figs. 1, 2) of chromosome 12 was present at 12p13.2–p13.32 on both chromosomes in 21 cells and only on one chromosome 12 in nine cells. No such double signal could be observed on any other chromosome.

## Discussion

In situ hybridization with a biotinylated genomic probe allowed us to localize the TNF-R1 gene at 12p13.2–p13.32. The TNFA and TNFB genes were previously localised at 6p21.3 (Spence et al. 1989). A number of genes have been localized to band 12p13 on the short arm of chromosome 12. Among them, some encode antigens like CD4 and CD9, others enzymes like enolase 2 (ENO2), glyceraldehyde-3-phosphate dehydrogenase (GAPD), and triose phosphate isomerase I (TPI1). The genes encoding the r and s subcomponents of complement component 1 (C1R and C1S) have also been localized at 12p13, while the gene encoding alpha-2-macroglobulin (A2M) occurs in the region 12p13.3–p12.3 (Ropers and Craig 1989).

The gene encoding the structurally related NGF receptor resides not on chromosome 12, but on chromosome 17. It would be interesting to find out whether any other member of the family to which the type I TNF-R belongs – and which have apparently all evolved from a common ancestor gene – localizes close to the type I TNF-R gene.

Bands 12p12–p13 are non-randomly involved in chromosomal rearrangements of malignancies such as acute lymphoblastic leukemia (Raimondi et al. 1986), acute myeloblastic leukemia (Berger et la. 1986) and malignant lymphoma (Jonveaux et al. 1991) either as "primary" or as secondary events. Possible rearrangements and deletions of the TNF-R1 gene remain to be investigated in these malignancies.

*Acknowledgements.* We thank Mr. B. Boursin for his photographic work, and Mrs. C. Czapek for her secretarial assistance. This work was supported by INSERM, the French Ministère de la Recherche et de la Technologie (Grant MRT No. 89 C 0878), the National Council for Research and Development (Israel), and the German Cancer Research Center.

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