Molecular Genetics of the Human GM-CSF Receptor

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Introduction

Granulocyte-macrophage colony stimulating factor (GM-CSF) is a glycoprotein of 23000 daltons which stimulates the proliferation, differentiation and functional activation of granulocytes, macrophages and eosinophils. Molecular clones encoding murine and human GM-CSF have been isolated and recombinant protein tested in vivo in various animal models. GM-CSF is also in advanced clinical trials to assess its efficacy as an agent for enhancing haemopoietic recovery and function in immunocompromised patients (for review, see [10]).

The biological activities of GM-CSF are transduced via specific cell-surface receptors. In both murine and human systems, autoradiographic analyses have indicated that GM-CSF receptors are present in low numbers (a few hundred per cell) on cells within the granulocyte/macrophage series. In addition, GM-CSF receptor have also been detected in various non-haemopoietic cells, including endothelial cells, small cell lung carcinoma cell lines, simian COS cells and placental cells (see [8], and references therein). Receptors of both high ($K_D \sim 30\,pM$) and low ($K_D \sim 1-3\,nM$) affinity have been detected [3, 4, 7, 18, 19, 24]. Chemical cross-linking studies have revealed multiple molecular species that can be cross-linked to radiolabelled GM-CSF [3, 4, 18, 24]. In murine systems, molecules of 180000, 130000, 70000 and 51000 daltons have been described, while in the human system, the reported molecular weights are 135000, 100000 and 80000. In some cases the higher molecular weight species have been correlated with high-affinity binding and the lower molecular weigth species with low-affinity binding [3].

We have recently cloned a complementary deoxyribonucleic acid (cDNA) encoding a human GM-CSF receptor with low binding affinity [8], and our recent biological, biochemical and molecular genetic studies on this receptor cDNA are summarized in this report.

Results and Discussion

Cloning of a Low-Affinity Human GM-CSF Receptor

A human GM-CSF receptor cDNA was cloned [8] by a direct expression strategy using a cDNA library prepared from placental RNA in the COS cell expressing vector πH3M (obtained from Dr. Brian Seed, Massachusetts General Hospital). The procedure we adopted utilized the extraordinary sensitivity and specificity of microscopic cell autoradiography to identify pools of cDNA that contained clones which could transfer the capacity to bind human GM-CSF to COS cells. Two independent cDNA clones, with identical coding regions, were isolated from approximately 5×10^6 clones screened (in 500 pools). The protein encoded by these cDNAs comprised an N-terminal hydrophobic leader sequence of

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22 amino acids, an extracellular domain of 297 amino acids, a single transmembrane segment of 27 non-polar residues and a short intracellular domain of 54 amino acids. The arrangement of four of the 11 cysteine residues in the extracellular domain as well as several other stretches of conserved amino acid sequence, notably the sequence motif Trp-Ser-X-Trp-Ser, identify the GM-CSF receptor as a member of a newly defined class of receptors [8], currently including those for growth hormone, prolactin, interleukins-2 (β chain), 3, 4, 6, 7, erythropoietin and granulocyte CSF. This cloned receptor does not bear any sequence homology with the protein tyrosine kinase family of receptors.

When introduced into COS cells, the cloned GM-CSF receptor cDNA directed the expression of large numbers (up to a million per cell) of receptors with the same low binding affinity ($K_D = 6$ nM) as found on placental tissue, and displaying a molecular weigth of about 85000 daltons [8].

Signal Transduction via the Low-Affinity Receptor

In order to determine whether the cloned low-affinity receptor could transduce a proliferative signal in haemopoietic cells, the receptor cDNA was introduced into murine FDC-P1 cells via a retroviral vector [15]. FDC-P1 cells are absolutely dependent upon murine GM-CSF or IL-3 for proliferation in vitro and express high-affinity murine GM-CSF receptors. FDC-P1 cells do not proliferate in response to human GM-CSF, which does not bind to murine receptors, even at very high concentrations.

After introduction of the human GM-CSF receptor, FDC-P1 cells displayed the same single class of low-affinity binding as seen on placental membranes and COS cells, and were able to proliferate in response to human GM-CSF [15]. The dose-response curve for human GM-CSF was, however, shifted to a 500 times higher dose than for murine GM-CSF, presumably reflecting the lower binding affinity of the human receptor.

Thus it was concluded that the cloned low-affinity receptor is able to deliver a proliferative signal in murine haemopoietic cells, and therefore, although there is no cross-species reactivity of murine and human GM-CSF, the human receptor is capable of interacting with the distal elements of the murine mitotic signalling pathway. Whether this receptor subunit is capable of transmitting a signal to induce differentiation remains to be determined.

Cross-Species Divergence of the GM-CSF Receptor Gene

The human GM-CSF receptor is encoded by a unique gene extending over about 50 kb of DNA (N. M. Gough, in preparation). In order to ascertain whether the human GM-CSF receptor cDNA could detect a murine homologue, Southern blots of murine and human genomic DNA were probed with various hGM-R cDNA probes at a range of stringencies. For example, in Fig. 1 at a stringency of 60 °C in 6 × SSC or above, no murine homologue was detected, and below this stringency (e.g. 55° C 6 × SSC) the level of background hybridization was too high for any cross-hybridization to be evident. In no experiments where hybridization conditions around this region were evaluated was any cross-hybridization to a murine homologue detected. A similar situation pertains for the ligand GM-CSF, in which no cross-hybridization between murine and human genes is perceptible on genomic Southerns (Fig. 2). These sequences display some 50% nucleotide sequence homology and cloned cDNAs clearly can cross-hybridize, albeit inefficiently, at reduced stringency (Fig. 3). Assuming that the rate of divergence of a hormone is restrained by the rate of divergence of its receptor, it would be likely that the murine and human GM-CSF receptors

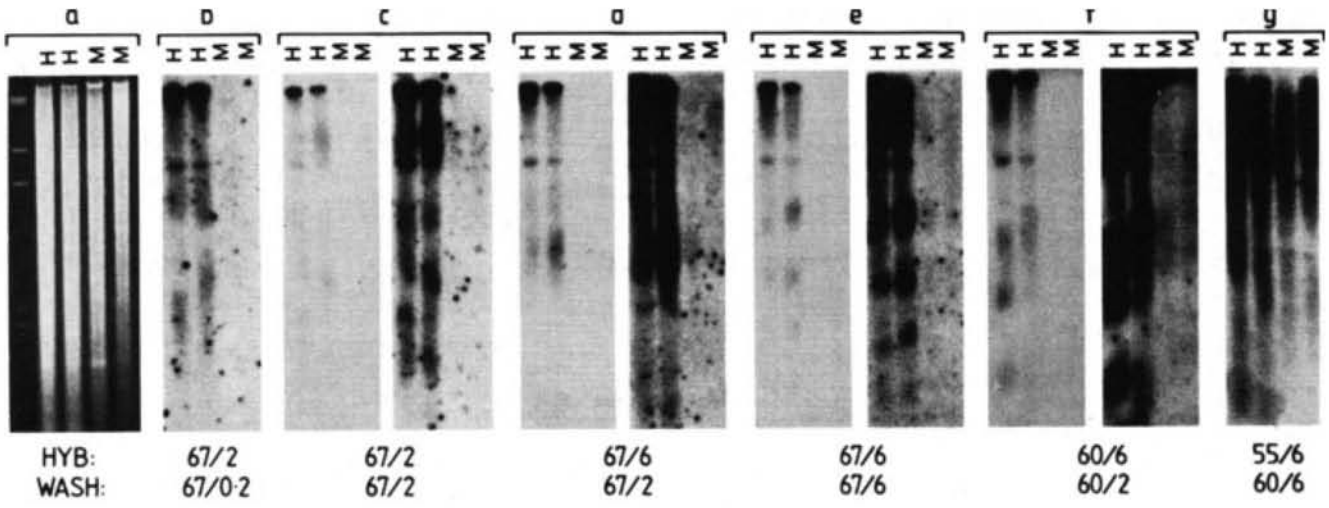

Fig. 1. Hybridization of human GM-CSF receptor cDNA to murine and human genomic DNA. DNA from peripheral blood of two normal human individuals (*H*) or two murine tissues (*M*) was digested with *Eco*RI and electrophoresed on 0.8% agarose gels. After transfer to nitrocellulose the DNA was hybridized with a probe corresponding to the region of the GM-CSF receptor cDNA clone pGMR138 located 5′ of the *Eco*RI site [8], under a variety of conditions of hybridization and washing (temperature and concentration of SSC given below the autoradiographs). General conditions for hybridization are described in [8]

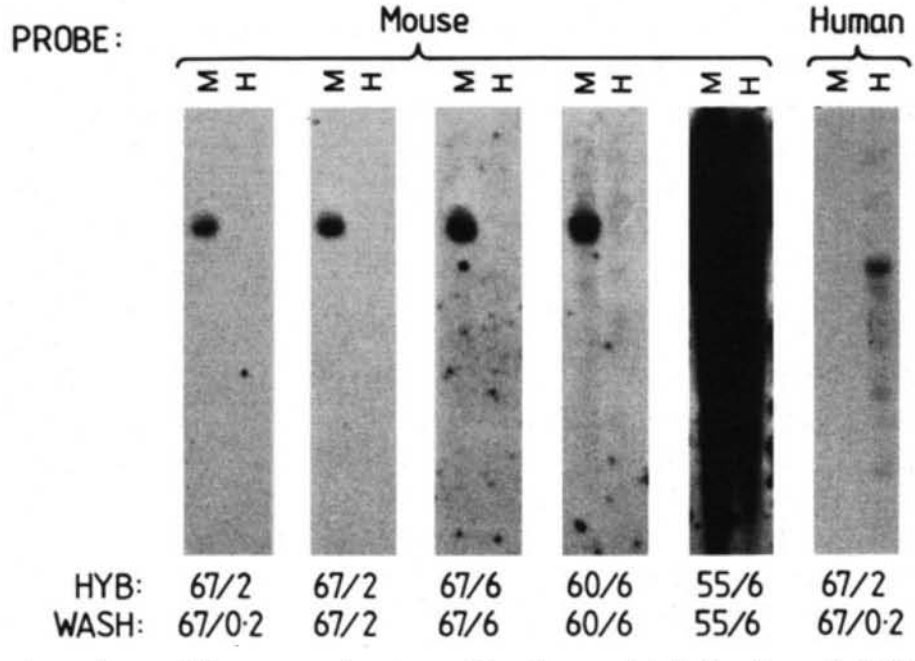

Fig. 2. Hybridization of murine and human GM-CSF cDNAs to murine and human genomic DNA. Normal human peripheral blood DNA (*H*) or murine DNA (*M*) was digested with *Hin*dIII and electrophoresed on 0.8% agarose gels. After transfer to nitrocellulose the DNA was hybridized with a murine (*left hand panels*) or human (*right hand panel*) GM-CSF cDNA probe, under a variety of conditions of hybridization and washing (temperature and concentration of SSC given below the autoradiographs). General conditions for hybridization are described in [8]

would have a similar degree of homology as for GM-CSF (approximately 50%) and would therefore not be detectable on genomic Southerns. Portions of the receptor which interact with the downstream signalling pathway have however presumably been conserved, since this human receptor is able to transduce a proliferative signal in murine haemopoietic cells (above).

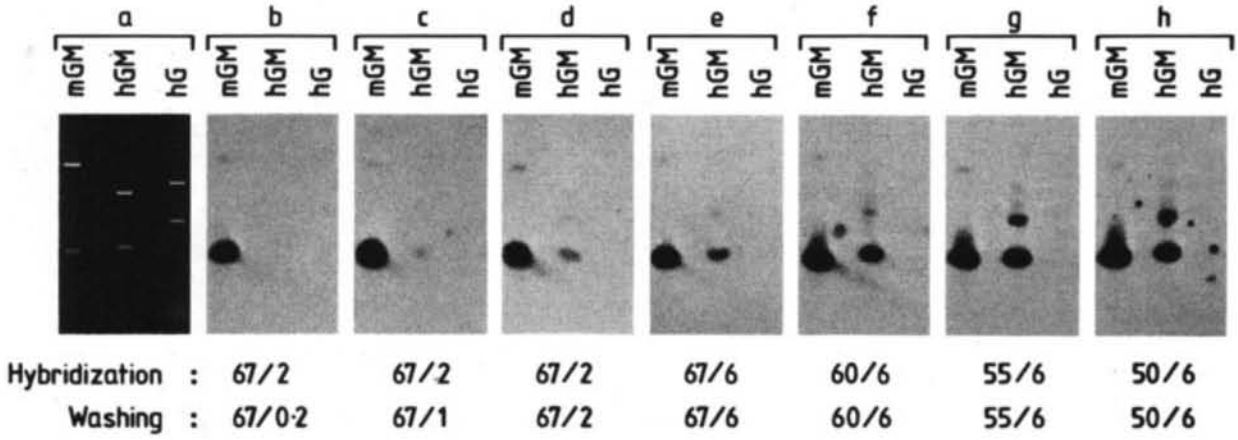

Fig. 3. Cross-hybridization of murine and human GM-CSF cDNAs. Plasmid DNA corresponding to a murine GM-CSF (*mGM*), human GM-CSF (*hGM*) and human G-CSF (*hG*) cDNA clones were digested to liberate the cDNA insert and electrophoresed on 1% agarose gels. After transfer to nitrocellulose the DNA was hybridized with a murine GM-CSF cDNA probe under a variety of conditions of hybridization and washing (temperature and concentration of SSC given below the autoradiographs). General conditions for hybridization are described in [8]

Pseudoautosomal Localization of the GM-CSF Receptor Gene

The human GM-CSF receptor gene was localized to the tip of the X and to the short arm of the Y chromosome by analysis of a panel of mouse-human somatic cell hybrids, RFLP analysis and in situ hybridization [11]. Localization of the GM-R gene to the tip of the sex chromosomes was consistent with it being within the pseudoautosomal region (PAR) [5]. This was formally proven by demonstration of exchange of alleles of the gene between the X and Y chromosomes during male meiosis. In three three-generation families studied, three exchanges of the GM-R gene between the sex chromosomes were found amongst 14 informative offspring, formally proving that GM-R locus is within the PAR. These data allowed a tentative location of the GM-R gene within the PAR to be deduced (see Fig. 4). The frequency of recombination of the GM-CSF receptor locus (20%) is clearly higher than for the MIC2 locus (2.5%), which has been mapped close to the PAR boundary, suggesting that the GM-R gene maps distal to this locus. The recombination frequency of the GM-R gene is similar to the frequencies observed for several anonymous pseudoautosomal loci, and the GM-R gene can be tentatively mapped between the loci DXYS15 and DXYS17 (Fig. 4). This localization is subject to more refined physical mapping studies, by pulsed field gel electrophoresis for example.

It has been long hypothesized that mammalian sex chromosomes have sequences in common which pair during male meiosis and allow exchange between the X and Y chromosomes (for review, see [5]). The existence of such a pseudoautosomal region was more recently directly demonstrated, and maps of the human PAR (a region of about 2500 kb) determined [5]. There is a great deal of interest in the nature of genes within this region and several candidates have been proposed, including genes associated with schizophrenia, cerebral dominance and Turner and Klinefelter syndromes [5]. MIC2, which maps just within the PAR, encodes a cell surface molecule implicated in T cell adhesion processes. The human GM-R gene is the first example of a gene of known function to be mapped to this region.

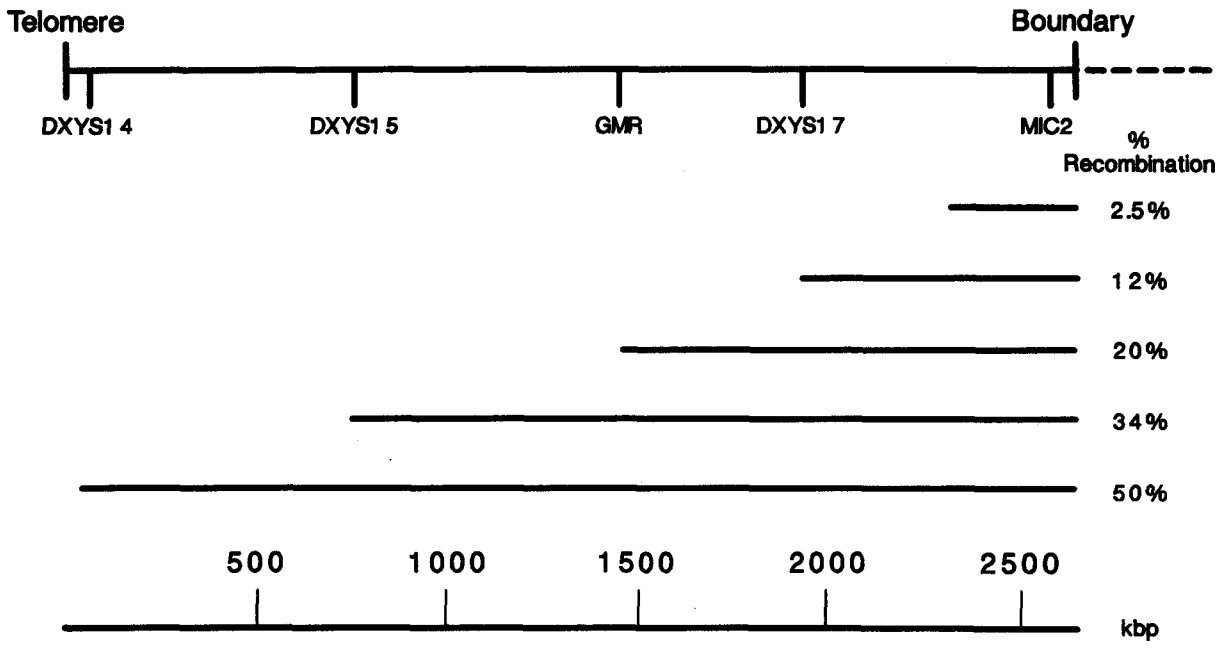

Fig. 4. Genetic map of the human pseudoautosomal region and localization of the human GM-CSF receptor gene. Recombination frequencies with respect to sexual phenotype are given on the *right*. Physical distance is given at the *bottom* in kb. (Data from [5])

Does the GM-CSF Receptor Gene Function as a Recessive Oncogene?

Loss of one or other of the sex chromosomes is frequent in certain acute myloid leukaemias, raising the possibility that loss of the GM-R gene may be involved in leukemogenesis. Loss of either the X or the Y chromosome is evident in 25% of acute myoblastic leukemias (AMLs) of the M2 subtype, compared with only 1%–6% in other AML subtypes [16] (Fig. 5a). Note that the X chromosome is lost from leukaemic cells of female patients with a very similar frequency to loss of the Y chromosome from leukaemias of males (Fig. 5b). The actual loss of the PAR (and hence the GM-R locus) may be significantly higher than 25%, since minor terminal chromosome deletions, which would be missed by standard karyotyping, have been described [20]. With the exception of chromosome 7, no other chromosome is lost to any significant extent in M2 AMLs [16]. It is important to note that loss of either the X or the Y chromosome occurs in 60%–65% of the subset of M2 AMLs characterized by the 8:21 chromosomal translocation (Table 1). Consistent chromosome loss is suggestive of the involvement of a "recessive oncogene" [21] in the genesis of M2 AML. Assuming that a single recessive oncogene is involved, then it is likely to be within the pseudoautosomal region, since if it were localized within a portion of the X chromosome not shared with Y (the majority of X) then similar loss of Y would not be predicted, and vice versa. That both chromosomes are involved implicates a gene shared between them, and hence most likely with the PAR. Thus, as has been described for the *Rb* gene implicated in the genesis of familial retinoblastoma [21, 25], it might be envisaged that gross cytological deletion of one allele of the putative oncogene would be associated with inactivation of the other allele. Although the PAR undoubtedly contains many genes that could be implicated, the GM-CSF receptor gene has the appropriate characteristics to be involved in the genesis of M2 AMLs. Loss or inactivation of both copies of this gene in a myeloid progenitor would generate a clone of cells unable to respond to GM-CSF, and hence possibly with a relatively

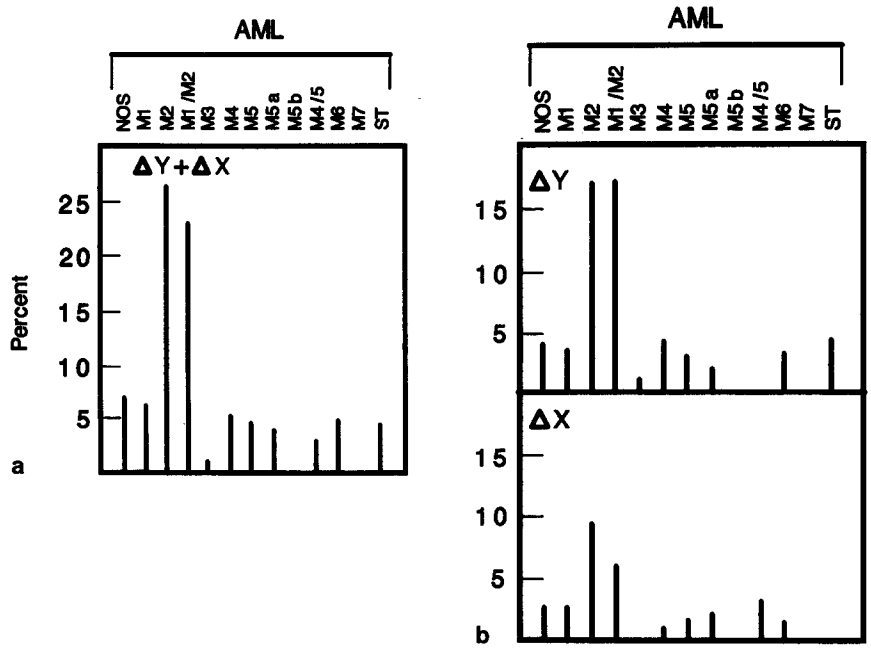

Fig. 5a, b. Loss of sex chromosomes in AML. The percentage of cases within each subtype of AML in which loss of either the X or the Y chromosome has been reported is plotted. The data for loss of either X or Y is compiled. The data are presented separately. (Data from [16])

undifferentiated phenotype, similar to that displayed by M2 AMLs. It is noteworthy that although most AMLs are responsive to GM-CSF, a significant subfraction are not [1, 2, 6, 17, 22, 23]. However, further studies are required to compare in more detail AML subtype, karyotype and GM-CSF receptor status. A second manner in which the GM-CSF receptor gene could function as an oncogene is in a semi-dominant fashion, analogous to the p 53 oncogene [13]. Thus an activating mutation on one allele may only be able to manifest itself in the absence of a wild type receptor, which might frequently be achieved by gross karyotypic deletion.

Table 1. Correlation of sex chromosome loss with t(8; 21) in M2 AML

Karyotype	Number of cases	
	M2 ($n=543$)	M1/M2 ($n=209$)
-X or -Y	25	14
-X or -Y/t(8; 21)	133	33
t(8; 21)	83	19

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