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25 March 1985; accepted 1 August 1985

The Human Gene Encoding GM-CSF Is at 5q21-q32, the Chromosome Region Deleted in the 5q⁻ Anomaly

Abstract. *Human granulocyte-macrophage colony-stimulating factor (GM-CSF) is a 22,000-dalton glycoprotein that stimulates the growth of myeloid progenitor cells and acts directly on mature neutrophils. A full-length complementary DNA clone encoding human GM-CSF was used as a probe to screen a human genomic library and isolate the gene encoding human GM-CSF. The human GM-CSF gene is approximately 2.5 kilobase pairs in length with at least three intervening sequences. The GM-CSF gene was localized by somatic cell hybrid analysis and in situ hybridization to human chromosome region 5q21-5q32, which is involved in interstitial deletions in the 5q⁻ syndrome and acute myelogenous leukemia. An established, human promyelocytic leukemia cell line, HL60, contains a rearranged, partially deleted GM-CSF allele and a candidate 5q⁻ marker chromosome, indicating that the truncated GM-CSF allele may reside at the rejoining point for the interstitial deletion on the HL60 marker chromosome.*

KAY HUEBNER
MASAHARU ISOBE
CARLO M. CROCE
The Wistar Institute, Philadelphia, Pennsylvania 19104
DAVID W. GOLDE
SUSAN E. KAUFMAN
JUDITH C. GASSON
Division of Hematology-Oncology, School of Medicine, University of California, Los Angeles 90024

Colony-stimulating factors (CSF's), proteins required for proliferation and differentiation of hematopoietic progenitor cells, are produced by a variety of human and murine cell types (*1*), including established cell lines (*2*). We previously described the purification of a 22,000-dalton glycoprotein with granulocyte-macrophage colony-stimulating activity (GM-CSF) from medium conditioned by the HTLV-II–infected human T-lymphoblast cell line Mo (*3*). Purified biosynthetic (recombinant) GM-CSF has all of the biological activities attributed to the protein purified from Mo-conditioned medium (*3, 4*). Differential staining of cells in bone marrow–derived colonies that had been stimulated by this GM-CSF demonstrated that the mediator induces growth of granulocyte, granulocyte-macrophage, macrophage, and eosinophil colonies (*5*). In addition, purified GM-CSF stimulates colony formation by the HL60 and KG-1 leukemic cell lines and is a weak inducer of differentiation in HL60 cells (*5*). Neutrophil migration in agarose is inhibited by purified natural and biosynthetic GM-CSF (*3*), and neutrophil oxidative metabolism is potentiated by GM-CSF (*6, 7*), suggesting an important role for this mediator in both the production and function of granulocytes for host defense.

High molecular weight DNA was prepared from the Mo (*8*) and J-WM-III (*9*) HTLV-II–infected T-lymphoblast cell lines, the K562 (*10*) human erythroleukemia cell line, and the WIL-2 human B-cell line and analysed (*11*) for hybridization to a GM-CSF–complementary DNA (cDNA) clone (*4*). Single fragments of approximately 8.0 kilobases (kb) and 5.2 kb were seen after digestion with Bam HI and Hind III, respectively. Fragments of 3.8 and 2.7 kb were detected after digestion with Bgl II. No differences in restriction fragment lengths were seen among DNA's from these cell lines, nor when Mo and human liver high molecular weight DNA's were compared (*4*). As digestion with Bgl II yielded two fragments that hybridize to the cDNA clone, which itself has no internal Bgl II sites, the GM-CSF gene contains at least one intron.

The GM-CSF gene was isolated from normal DNA in order to determine its structural organization. A recombinant phage library (*12*) (prepared from Charon 4a and fetal liver DNA) was screened with the GM-CSF cDNA clone as a probe. Two genomic GM-CSF clones were obtained from approximately 750,000 recombinant phage. The clones each contained the Bam HI, Hind III, and both Bgl II restriction fragments detected by DNA hybridization analysis (Fig. 1, CSF-2; the map was the same for both clones). Further comparative restriction enzyme mapping of the genomic and cDNA clones revealed the existence of at least three introns in the human GM-CSF gene (Fig. 1). The total length of the gene is approximately 2.5 kb.

A panel of 25 mouse-human hybrids (*13*), retaining defined subsets of human chromosomes, was analyzed for the presence of the GM-CSF gene by hybridization of their DNA with the genomic clone of the 5.2-kb Hind III fragment as a probe (pCH5.2, Fig. 1). After hybridization of pCH5.2 plasmid DNA to Hind III–digested DNA from a normal donor, a single band of approximately 5.2 kilobase pairs (kbp) was seen, while mouse

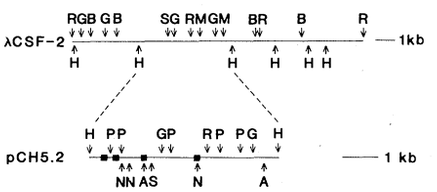

Fig. 1. Genomic map of the human GM-CSF gene. A bacteriophage λ recombinant library (*12*) was propagated on DP50 supf bacteria with ~25,000 recombinant phage per 100-cm plate. The library was screened by plaque hybridization with the full-length gel-purified GM-CSF cDNA clone that had been labeled with ^{32}P by nick translation. Restriction maps were obtained by digesting DNA with various restriction enzymes, fractionating the digests on agarose gels, transferring the DNA to nitrocellulose filters, and hybridizing the filters with the ^{32}P-labeled GM-CSF cDNA insert. The 5.2-kb Hind III fragment of the CSF-2 that hybridized to the GM-CSF cDNA clone was subcloned into the Hind III site of pBR322 (pCH 5.2). In the construction of restriction maps for the subclone, the hybridization probe consisted of two smaller fragments of the GM-CSF cDNA clone that were prepared with Apa I and Eco RI, gel purified, nick translated, and hybridized individually to restriction endonuclease–digested pCH5.2 DNA. The fragments generated by digestion of the GM-CSF cDNA insert with Apa I represent nucleotides 1 to 288 (5′) and 289 to 780 (3′). The sites of cleavage for Eco RI (R), Sal I (S), Sma I (M), Hind III (H), Bgl II (G), Bam HI (B), Apa I (A), Nco I (N), and Pvu II (P) are shown.

control DNA contained a single higher molecular weight band (Fig. 2, lanes 1 and 2). Eight of the 17 hybrid cell lines were positive for the 5.2-kbp GM-CSF gene fragment. These hybrids and an additional eight were also tested for presence of the GM-CSF gene after digestion with Bam HI and Eco RI; hybridization occurred to fragments of the expected size.

The somatic cell hybrid analysis indicated that the presence of the GM-CSF gene was correlated with the presence of human chromosome 5 in the hybrids (Table 1). Hybrid DNA's were also analyzed with a c-*fms* probe (*14*) to confirm the presence or absence of chromosome 5 in the cells. The human c-*fms* homolog of v-*fms* has been mapped to region 5q34 (*15*). Somatic cell hybridization analysis was only consistent with the localization of the GM-CSF gene to chromosome 5.

To confirm and refine the somatic cell hybrid results, in situ hybridization to human metaphase chromosomes was done with pCH5.2. After autoradiography, more than 32 percent of all grains were located on the long arm of chromosome 5. More than 95 percent of 5q grains were between 5q21 and 5q32, with most grains at 5q23 (Fig. 3). The long arm of chromosome 5 represents approximately 4.5 percent of the haploid genome, and our observation that more than 30 percent of the human GM-CSF probe hybridization was localized to the distal half of this region is highly significant by the χ^2 test ($P < 0.001$). Thus, cytological hybridization localized the

Table 1. Correlation of presence of GM-CSF gene and specific human chromosomes in 25 mouse-human hybrids. A panel of hybrid cells (*13*) was characterized for the presence of specific human chromosomes by isozyme analysis and, in some cases, karyotypic analysis and DNA:DNA hybridization with DNA probes for genes assigned to specific chromosomes. The DNA was analyzed for the presence of the human GM-CSF gene as described in Fig. 2.

Human chromosomes	Number of hybrid clones (GM-CSF gene/chromosome retention)				Discordance (%)
	+/+	−/−	+/−	−/+	
1	6	10	7	2	36
2	2	11	11	1	48
3	11	10	2	2	16
4	9	7	4	5	36
5	13	12	0	0	0
6	6	10	7	2	36
7	5	5	8	7	60
8	6	9	7	3	40
9	6	8	7	4	44
10	7	12	6	0	24
11	3	9	10	3	52
12	2	7	11	5	64
13	7	8	6	4	40
14	11	5	2	7	36
15	4	8	9	4	52
16	1	10	12	2	56
17	9	6	4	6	40
18	5	9	8	3	44
19	4	9	9	3	48
20	7	8	6	4	40
21	2	9	11	3	56
22	8	8	5	4	36
X	10	10	3	2	20

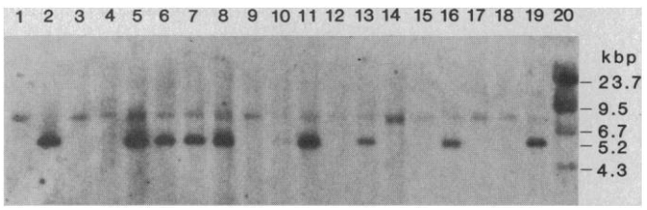

Fig. 2 (left). Somatic cell hybrid DNA panel probed with GM-CSF plasmid pCH5.2. Lanes 1 and 2 represent DNA from the murine cell line and from normal human peripheral blood lymphocytes (PBL), respectively. Hybrids retained the following human chromosomes: (lane 3) chromosome 7; (lane 4) chromosomes 6, 7, 17, and 21; (lane 5) chromosomes 1, 3–10, 13, 14, 17, 20, 22, and X; (lane 6) chromosomes 1, 3–10, 13, 14, 17, 18, 20, 22, and X; (lane 7) chromosomes 5, 8, 14, 15, 17–19, 21, 22, and X; (lane 8) chromosomes 3–5, 14, 15, 17, 20, 22, and X; (lane 9) chromosomes 4, 7, 11, 12, 14–17, 20 and 22; (lane 10) chromosomes 4, 6, 7, 13, 14, 17, 18, and 20; (lane 11) chromosomes 3–5, 9, 10, 13, 14, 17, 20, 22, and X; (lane 12) chromosomes 1–4, 6–9, 11, 12, 14, 15, 18, 20, 21, and X; (lane 13) chromosomes 1, 3–6, 10, 11, 14, 17, and X; (lane 14) chromosome 17; (lane 15) chromosome 14q$^+$ from a Burkitt lymphoma; (lane 16) chromosomes 2, 3, 5, 12, 17, 18, and 20; (lane 17) chromosomes 9, 12–14, 17, and 22; (lane 18) chromosomes 9, 12–14, 17, 21, and 22; (lane 19) chromosomes 5, 7–9, 12–15, 17, 21, and 22. DNA (10 μg/lane) was digested with Hind III, fractionated electrophoretically in agarose, and transferred to a nitrocellulose filter; the filter was hybridized to nick-translated ^{32}P-labeled pCH5.2 plasmid DNA as described (*13*). Lane 20 contains 1 μg of Hind III–cleaved bacteriophage λ DNA markers. Sizes of markers and the human GM-CSF fragment (5.2 kbp) in lanes 2, 5–8, 11, 13, 16, and 19 are given on the right of the figure.

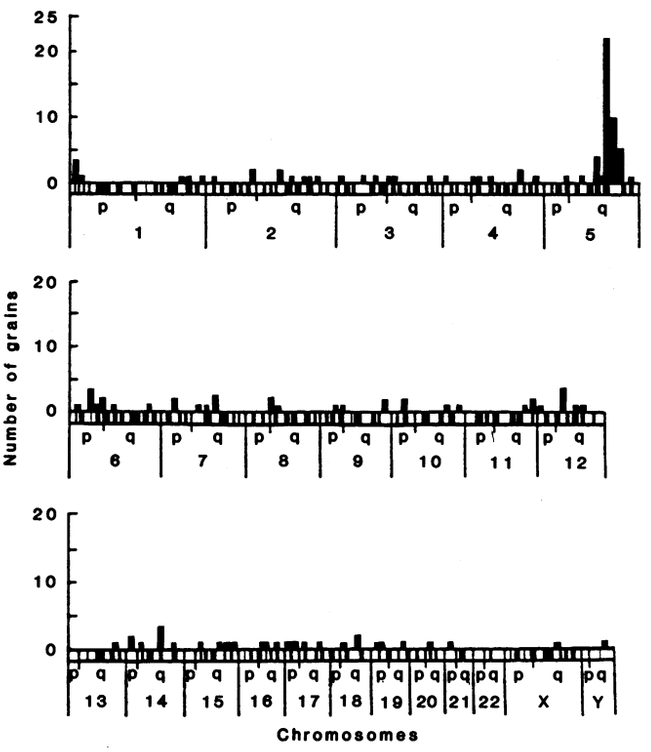

Fig. 3 (right). In situ hybridization of GM-CSF pCH5.2 plasmid DNA to normal human metaphase chromosomes: The abscissa represents the chromosomes in their relative size proportions. The distribution of 139 grains on 100 spreads was scored; 42 were found over 5q21-5q32. Metaphase spreads were prepared with normal human male lymphocyte cultures that had been phytohemagglutinin-stimulated in vitro for 72 hours. The pCH5.2 DNA was nick translated with [^{3}H]dCTP (62 Ci/mmol), [^{3}H]dGTP (39.9 Ci/mmol), [^{3}H]dTTP (100.1 Ci/mmol), and [^{3}H]dATP (51.9 Ci/mmol). In situ hybridization and G-banding were essentially as described (*21, 22*).

human GM-CSF gene to the region between 5q21 and 5q32.

A novel restriction fragment pattern was observed when DNA from the HL60 (*16*) promyelocytic leukemia cell line was examined with pCH5.2 as probe and the enzymes Hind III and Eco RI (Fig. 4), as well as Bgl II. The results are consistent with the presence of one normal GM-CSF allele and one truncated allele (2.5-kbp fragment, Fig. 4A, lane 2; 8.0-kbp fragment, Fig. 4A, lane 3). Further analysis of HL60 DNA with the GM-CSF cDNA insert as a probe did not detect the novel restriction fragments generated by Hind III (Fig. 4B, lane 14), Eco RI, or Bgl II. These results demonstrate a deletion of one GM-CSF gene encompassing most or all of the coding region in HL60. The data suggest that the end point of the deletion is on the 3′ side of the Eco RI restriction site shown in the pCH5.2 genomic clone in Fig. 1.

The localization of the human GM-CSF gene to chromosome region 5q21-5q32 is important from several perspectives. The 5q$^-$ chromosome, involving acquired deletions in the long arm of chromosome 5, was originally reported as the only chromosomal abnormality in three patients with refractory anemia (*17*). This disorder became known as the 5q$^-$ syndrome. The syndrome is generally seen in older women with refractory macrocytic anemia, normal or slightly reduced leukocyte count, and elevated platelet counts. The same chromosomal anomaly has often been detected in patients with acute myelogenous leukemia (AML), usually in association with other nonrandom chromosome abnormalities (*18*). In addition to partial 5q deletions in refractory anemia and leukemia, monosomy for chromosome 5 has also been described in AML and is a frequent abnormality in patients with AML who have a history of exposure to toxic chemicals (*19*). The limits of the 5q$^-$ interstitial deletions have been reported variously to be from 5q11 to 5q23 (for the proximal breakpoint) to 5q22 to 5q33 or 34 (for the distal breakpoint) (*17*). Although there was no complete overlap between all deletions, the region of overlap is probably near 5q21-5q23, with the shortest region of overlap being in 5q22 (*17*).

Although the 5q$^-$ chromosome abnormality seems to affect a stem cell common to the granulocytic, erythroid, and megakaryocytic lineages, no explanation to connect the specific deleted chromosome region with the respective hematologic disorders has been forthcoming. We believe that the localization of the GM-CSF locus to a region that is deleted in most, if not all, of the reported 5q$^-$ deletions provides a starting point to link the various hematological disorders with specific genetic lesions. The position of the GM-CSF gene on chromosome 5 puts it close to the smallest consistent deletion seen in 5q$^-$ cases.

The HL60 cell line is monosomic for normal chromosome 5 (*16, 20*), and has a marker chromosome (M3) that might include a part of chromosome 5 (*20*). The Southern analysis of the HL60 GM-CSF locus indicates that HL60 must retain at least part of a partially deleted long arm of chromosome 5. Another explanation for this data and the results of the karyotype analysis would be that the HL60 M3 chromosome is a 5q$^-$ chromosome in which the interstitial deletion resulted from breakage of the long arm of chromosome 5 within the GM-CSF locus, resulting in loss of the deleted portion of chromosome 5.

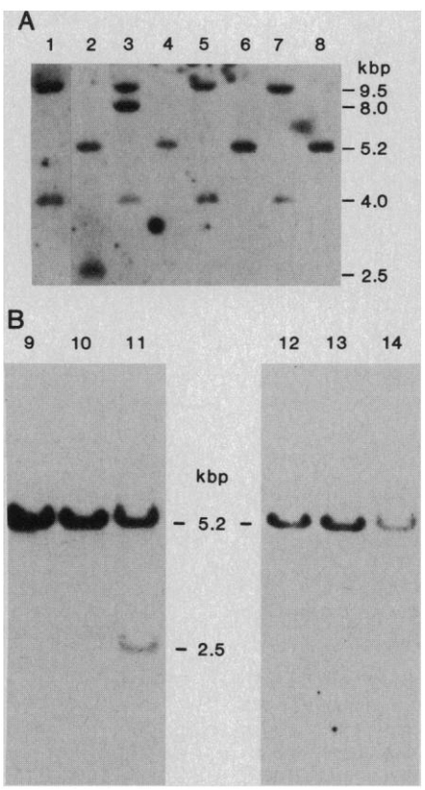

Fig. 4. A GM-CSF allele is rearranged in HL60 cells. (A) DNA from normal human PBL (lane 1); HL60 cell line (lanes 2 and 3); K562 cell line (lanes 4 and 5); ML3 cell line (*23*) (lanes 6 and 7); GM6141 cell line (*23*) (lane 8). The DNA's were cleaved with Eco RI (lanes 1, 3, 5, and 7) or Hind III (lanes 2, 4, 6, and 8) and processed as described in Fig. 2. The filter was hybridized to ^{32}P-labeled GM-CSF plasmid pCH5.2. Sizes of all GM-CSF fragments detected by this probe are shown on the right of the figure. (B) DNA samples from the Mo-T lymphoblast (lanes 9 and 12), K562 erythroleukemia (lanes 10 and 13), and HL60 promyelocytic leukemia (lanes 11 and 14) cell lines were digested with Hind III, fractionated on an agarose gel, and transferred to nitrocellulose. The nitrocellulose filter was divided in half and hybridized to the pCH5.2 probe (lanes 9–11) or the full-length GM-CSF cDNA clone, gel-purified, and labeled with ^{32}P by nick translation.

The rearrangement and truncation of the GM-CSF locus in the established cell line HL60 may be useful in the dissection of the deleted locus, especially if the M3 chromosome is indeed a 5q$^-$ chromosome. Many genes must be deleted in these 5q interstitial deletions, and the deleted genes will not necessarily be common for all described partial deletions of 5q. While complete deletion of genes is not easily detected in human bone marrow–derived DNA, because of the presence of one normal chromosome 5, rearrangements such as that in HL60 cells are easily observed. Therefore, it will not be difficult to determine if the GM-CSF locus is frequently rearranged in DNA derived from bone marrow of patients exhibiting a 5q$^-$ chromosome.

Note added in proof. Neinhuis *et al.* (*24*) have recently reported that the human c-*fms* proto-oncogene is deleted in the 5q$^-$ syndrome.

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18 July 1985; accepted 26 September 1985

Direct Evidence That Endogenous G_{M1} Ganglioside Can Mediate Thymocyte Proliferation

Abstract. *The B subunit of cholera toxin, which is multivalent and binds exclusively to a specific ganglioside, G_{M1}, was mitogenic for rat thymocytes. When exposed to the B subunit, the cells proliferated, as measured by ^{3}H-labeled thymidine incorporation. Mitogenesis depended on the direct interaction of the B subunit with G_{M1} on the surface of the cells. This demonstrates that endogenous plasma membrane gangliosides can mediate proliferation in lymphocytes.*

SARAH SPIEGEL
PETER H. FISHMAN
Membrane Biochemistry Section, Developmental and Metabolic Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205
RICHARD J. WEBER
Neuroimmunology Unit, Section on Brain Biochemistry, Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda

Various cellular recognition phenomena and some important biological functions have been ascribed to glycolipids, particularly the sialoglycosphingolipids or gangliosides. Although gangliosides are receptors for bacterial toxins (*1*) and viruses (*2*) and can function as tumor antigens (*3*), their normal functioning in the plasma membrane remains unknown. Gangliosides can suppress the immune response in vitro (*4*) and can inhibit the growth of fibroblasts (*5*). In the latter case, the added gangliosides inhibited the interaction of growth factors with their receptors as well as the receptor-associated tyrosine kinase activity. On the other hand, gangliosides seem to stimulate cell growth and differentiation. The ganglioside G_{Q1b}, when added to certain neuroblastoma cell lines, increases both cell number and the length and number of neurites (*6*). Gangliosides, especially G_{M1}, promote neuritogenesis in both primary neurons and neuroblastoma cells (*7*). When hapten-modified gangliosides are inserted into thymocytes, the cells can proliferate in response to multivalent hapten-binding proteins (*8*). The researchers conducting these studies, however, added exogenous gangliosides to elicit the observed effects and did not address the possible functions of endogenous gangliosides. We have found that thymocytes can be induced to proliferate in response to the B subunit of cholera toxin (CT), which binds specifically to the endogenous ganglioside G_{M1} (*1*).

Exposure of rat thymocytes to the B subunit increased incorporation of [^{3}H]-thymidine relative to that of media-treated controls (Table 1). A concentration of B subunit as low as 25 ng/ml (~0.5 n*M*) induced proliferation, which reached a plateau at higher concentrations. Although the stimulation induced varied among experiments, so did that induced by concanavalin A (Con A) (Table 1). The relative responses to the two effectors, however, were of the same magnitude and were comparable between different experiments. Optimal incorporation of [^{3}H]thymidine was observed when cultures were pulsed from 48 to 64 hours after stimulation with the B subunit. The best results were obtained with thymocytes from Sprague-Dawley rats raised under germ-free conditions (Zivic-Miller Labs, Allison, Pennsylvania). In contrast, thymocytes from conventional Sprague-Dawley or Wistar rats responded moderately (fourfold) and thymocytes from C3H/HEN mice did not respond.

To confirm that the observed stimulatory activity was due to the binding of the B subunit to the cells, we incubated the subunit with antibodies to CT. The increase in thymidine incorporation observed was inhibited by the antibodies in a dose-dependent manner; 1:100 and 1:10 dilutions inhibited 40 and 100 percent, respectively (*9*). To further confirm the interaction of the B subunit with the thymocytes, we measured iodinated CT binding to the cells and its inhibition by the B subunit and the antibodies to CT (*10*). Under saturating conditions, 21 fmol of ^{125}I-CT were specifically bound per 10^6 cells. Binding of ^{125}I-labeled CT was completely inhibited (>97 percent) by the addition of unlabeled CT or subunit B or the antibodies. These data make it seem likely that the mitogenic effects of subunit B are due to its specific binding to the cell surface receptor for CT (that is G_{M1}) and that the inhibition of these effects by the antibodies is due to

Table 1. Stimulation of [^{3}H]thymidine incorporation into rat thymocytes by the B subunit. Rat thymocytes were prepared (*8*), washed, and maintained in RPMI 1640 medium supplemented with 5 percent fetal bovine serum, 1 m*M* sodium pyruvate, nonessential amino acids, 2 m*M* L-glutamine, penicillin (100 unit/ml), and streptomycin (100 μg/ml). The thymocytes (2×10^6 per well) were cultured in flat-bottomed microtiter trays in 0.2 ml of medium with the indicated concentrations of Con A or B subunit (Calbiochem-Behring or List Biological). After 48 hours, the cells were pulsed with 1 μCi of [^{3}H]thymidine for 16 hours, collected, and assayed for [^{3}H]thymidine incorporation. Each value is the mean of triplicate wells and each experiment used a different preparation of thymocytes.

Mitogen (μg/ml)	[^{3}H]Thymidine incorporation (counts/min ± S.E.M.)			
	Experiment 1	Experiment 2	Experiment 3	Experiment 4
B subunit				
0	3440 ± 273	1690 ± 199	3775 ± 381	483 ± 200
0.025	2770 ± 497	–	–	28400 ± 3140
0.25	10750 ± 1640	–	25200 ± 4625	49300 ± 2450
2.5	1 2400 ± 1410	37900 ± 1805	21460 ± 2160	50050 ± 4630
25.0	16200 ± 2540	40100 ± 667	23700 ± 445	62300 ± 4350
Con A				
2	194000 ± 7650	–	147000 ± 14600	252000 ± 6630