

# Decreased expression of FcγRIII mRNA in leukemic granulocytes

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Received 13 January 1992

Morphologically mature granulocytes from patients with chronic myeloid leukemia show significant impairment in their ability to internalize aggregated IgG, a ligand that is rapidly phagocytosed by normal human granulocytes. With a view to understand the molecular basis of this defect, normal and leukemic granulocytes were examined for the steady-state levels of mRNA for FcγRIII, a membrane-associated receptor that initially binds and traps the IgG-opsonized antigens. Northern blot analyses revealed that the level of the specific mRNA in CML granulocytes was between 0.08 and 0.69 times that seen in the normal granulocytes. This could be one of the contributory factors for the observed endocytic defect in the leukemic granulocytes.

FcγRIII; Leukemic; Granulocyte

## 1. INTRODUCTION

Human neutrophils express two classes of Fcγ receptors which mediate the binding and subsequent ingestion of IgG-opsonized bacteria. FcγRII is a 40 kDa transmembrane protein, while FcγRIII is a 50–70 kDa molecule linked to the membrane via a PI-tail [1]. Recent studies have shown that FcγRIII, present in greater number ( $1.35 \times 10^5$  sites/cell), is required for the initial trapping and binding of the opsonized particles, while FcγRII is involved in the subsequent signal transduction and phagocytosis of the bound particle [2]. Deficiency of any of these receptors on the cell surface could reduce the phagocytic ability of granulocytes and render the host prone to infection.

Morphologically mature granulocytes from patients with chronic myeloid leukemia (CML), exhibit several biochemical and functional defects. One of these is their impaired ability to internalize aggregated IgG, a ligand efficiently phagocytosed by the normal human granulocytes [3]. This defect could arise due to change(s) in: (a) the structure/amount of FcγR on the cell surface, (b) its association with the underlying cytoskeleton, and (c) subsequent transmembrane signalling events.

With a view to understand the molecular basis of this defect, we have investigated the steady-state levels of FcγRIII mRNA in normal and CML granulocytes.

## 2. MATERIALS AND METHODS

### 2.1. Preparation of cells

Peripheral blood from normal voluntary donors was collected in acid-citrate-dextrose solution, while that from untreated CML patients (with WBC counts of  $100\text{--}450 \times 10^9/l$ ) was collected in heparin (10 IU/ml). Morphologically mature granulocytes from each sample, were recovered from the pellet after Ficoll-Hypaque sedimentation [4] of the respective buffy coats. Mononuclear cells and immature myeloid precursors, which accumulated at the interphase, were also collected from the normal and CML samples respectively. After lysing the contaminating RBCs with 0.83% buffered ammonium chloride, the cells were washed twice with ice-cold PBS (136 mM sodium chloride, 1 mM potassium chloride, 3 mM disodium hydrogen orthophosphate, 70 mM potassium dihydrogen orthophosphate, pH 7.3). The pelleted fractions from normal and CML samples contained an average 99% and 90% viable granulocytes, respectively.

### 2.2. Northern blotting

RNA was isolated according to the single step method of Chomczynski and Sacchi [5]. Twenty μg of total RNA from each sample were fractionated on a 1.8% agarose gel containing 2.2 M formaldehyde, according to Maniatis [6]. The separated RNAs were transferred to Hybond N membranes (Amersham) essentially as described in the accompanying instructions [7] using 20×SSPE (3 M NaCl, 200 mM NaH<sub>2</sub>PO<sub>4</sub>, 20 mM EDTA, pH 7.4). The FcγRIII transcript was detected using the pGP5 clone for FcγRIII [8]. The human catalase transcript was detected using the 0.8 kb insert from a pSP64 clone following HindIII and EcoRI digestion.

The FcγRIII plasmid DNA was labelled with [ $\alpha$ -<sup>32</sup>P]dCTP (BRIT, India) by nick translation using the BRL nick translation kit, while the catalase insert DNA was labelled using the Multiprime labelling kit purchased from Amersham, UK. Specific activities of  $10^7\text{--}10^8$  cpm/μg were obtained.

The filters were prehybridized for 1 h at 42°C in 50% formamide, 5×SSPE, 5× Denhardt's solution, 0.5% SDS w/v, 100 μg/ml denatured, sonicated Salmon sperm DNA and then hybridized overnight at 42°C in the same solution containing  $10^7\text{--}10^8$  cpm/ml of the denatured probe. The filters were washed twice with 2×SSPE/0.1% SDS for 15 min at 65°C and once with 1×SSPE/0.1% SDS for 30 min at 65°C. They were Saran-wrapped and exposed to Fuji X-ray film between two Lightning Plus Dupont Cronex intensifying screens at –70°C.

The intensities of the bands on the autoradiographs were measured

**Abbreviations:** CML, chronic myeloid leukemia; PI, phosphatidyl-inositol; PNH, paroxysmal nocturnal hemoglobinuria.

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using a Densitometer (EC Apparatus Corporation, USA) equipped with a Hewlett Packard 3390 A Integrator.

### 3. RESULTS

The steady-state levels of the mRNA for Fc $\gamma$ RIII in normal and CML granulocytes, the mononuclear cells from normal blood and immature myeloid precursors from CML blood were analysed by Northern blotting. The integrity and amount of RNA loaded were ascertained using the catalase probe, since the catalase gene is constitutively expressed and it has been shown that the signals on Northern blots are proportional to the ethidium bromide staining [9].

Fig. 1 shows the ethidium bromide-stained RNA profiles on agarose gels and the autoradiographic profiles of the corresponding blots probed with the catalase cDNA and the Fc $\gamma$ RIII clone. It can be seen that the mRNA for Fc $\gamma$ RIII is present in normal and CML granulocytes, but it is absent in the mononuclear cells and the immature myeloid precursors. The intensities of the bands in the normal and CML granulocyte RNA lanes reveal that the expression in the latter cell population is very low. This difference was quantitated by measuring the ratios of the intensities of the Fc $\gamma$ RIII signal to the catalase signal, for each lane and comparing the value thus obtained, for the normal and CML samples, Table I. It is seen that the Fc $\gamma$ RIII signals for the CML granulocyte samples, three in blot A, two in B and two in C are lower than the corresponding normal samples on each blot.

### 4. DISCUSSION

CML is a clonal hematological disorder characterized by discordant maturation of the myeloid pre-

Table I  
Comparison of the steady-state levels of the Fc $\gamma$ RIII mRNA in normal and CML granulocytes

Northern blots	Sample	Fc $\gamma$ RIII/Catalase*	% Granulocytes
A	N <sub>1</sub> G	1.0	99%
	N <sub>2</sub> G	0.98	99%
	N <sub>3</sub> G	1.22	99%
	C <sub>1</sub> G	0.31	91%
	C <sub>2</sub> G	0.14	83%
	C <sub>3</sub> G	0.14	90%
B	N <sub>4</sub> G	1.00	99%
	C <sub>4</sub> G	0.23	90%
	C <sub>5</sub> G	0.08	91%
C	N <sub>4</sub> G	1.00	99%
	C <sub>6</sub> G	0.69	90%
	C <sub>7</sub> G	0.56	89%

\*Ratio of the intensities of the Fc $\gamma$ RIII and Catalase signals from the corresponding blots.

N<sub>1</sub>G-N<sub>4</sub>G and C<sub>1</sub>G-C<sub>7</sub>G refer to the different normal and CML granulocyte samples, respectively.

cursors [10]. This results in circulation, in the peripheral blood of morphologically mature but functionally immature granulocytes which are unable to internalize soluble aggregated IgG [3]. To investigate whether this defect was due to an abnormal expression of the receptor for IgG, the steady levels of mRNA for Fc $\gamma$ RIII were measured. Our data shows that the mRNA for the molecule is present in reduced amounts (0.08–0.69 times that of normal) in leukemic granulocytes. The lowered amounts may be due to a decreased rate of transcription or due to the instability of the transcript. This may be one of the causes for the observed defective endocytosis of aggregated IgG.

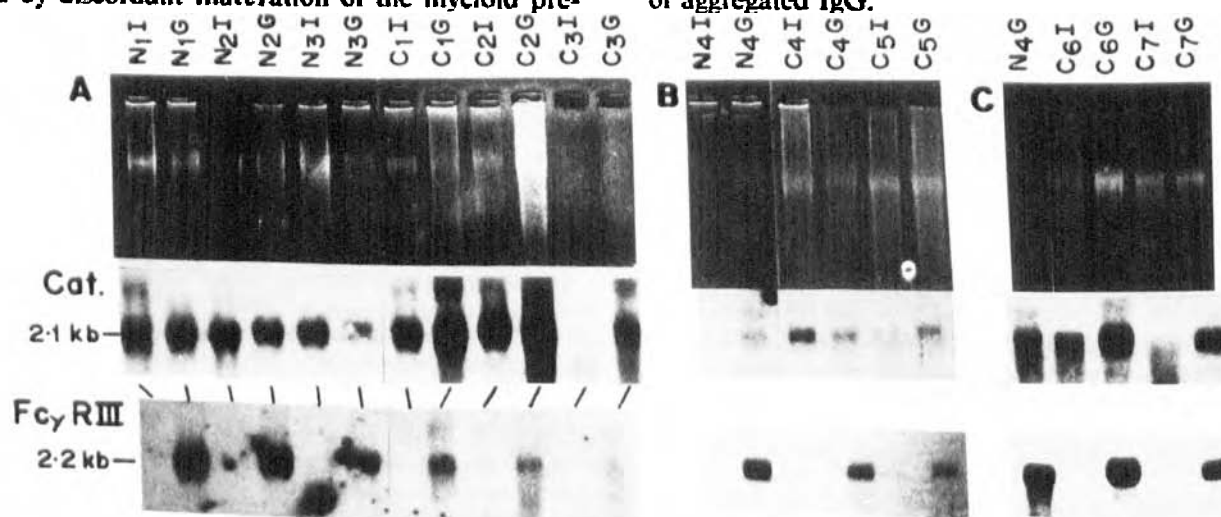


Fig. 1. Northern blotting of RNA from normal and CML granulocytes, mononuclear cells and immature myeloid precursor cells from normal and CML peripheral blood, respectively. A, B, C show the 1.8% agarose gels stained with ethidium bromide and the autoradiographs of their corresponding blots probed with Catalase (Cat.) and Fc $\gamma$ RIII clones. C<sub>1</sub>I and C<sub>1</sub>G refer to the immature myeloid cells and granulocytes, respectively, from different CML patients; N<sub>1</sub>I and N<sub>1</sub>G refer to the mononuclear cells and granulocytes, respectively, from different normal individuals.

We have confirmed the underexpression, by calculating the FcγRIII mRNA signal relative to the catalase mRNA signal. The expression of the FcγRIII protein has been shown to begin at the metamyelocyte stage with the levels increasing as the cell matures to the granulocytic stage [11]. Since immature myeloid precursors from CML cells do not express the RNA for the protein, its lowered expression in the morphologically mature CML granulocytes, suggests that these cells may be 'frozen' in an earlier stage of differentiation with respect to this molecule. This lowered expression cannot be attributed to contamination from myeloid precursors as the decrease is not proportional to the percentage of immature cells in the granulocyte preparations (Table I).

In an earlier study, Rambaldi et al. [12] have reported that the mRNA for leukocyte alkaline phosphatase is absent in CML granulocytes. This protein is also a PI-tailed protein like the FcγRIII. The lowered expression of these two PI-linked proteins in CML seems to follow the trend that is observed with respect to the decreased expression of PI-linked proteins, leukocyte alkaline phosphatase, acetylcholine esterase, FcγRIII and decay acceleration factor in PNH patients [13–16]. In the latter disorder however, the lowered expression of these proteins is believed to be associated with a deficiency in glycosyl-phosphatidylinositol synthesis or its transfer to the protein [17] and not due to decreased levels of the mRNA for these molecules [12,18]. It is most probable that in CML cells too, there is a common mechanism which controls the steady levels of the mRNA for these PI-linked proteins.

**Acknowledgements:** This work was supported by a grant from the Department of Science and Technology, India. Joanita Monteiro is a Senior Research Fellow supported by the Council of Scientific and

Industrial Research, India. We would like to acknowledge Dr. Kevin W. Moore, DNAX Research Institute, California, USA for providing us with the FcγRIII cDNA clone.

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