

Basic Medical Research

# IFN $\gamma$ modulates human immunoglobulin receptor expression in lipoaspirate-derived mesenchymal stem cells

Dian R. Laksmiawati,<sup>1</sup> Jeanne A. Pawitan,<sup>2</sup> Mohamad Sadikin,<sup>3</sup> Caroline T. Sardjono,<sup>4</sup> Ahmad R. Utomo<sup>5</sup>

<sup>1</sup> Faculty of Pharmacy, Pancasila University, Jakarta, Indonesia

<sup>2</sup> Department of Histology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

<sup>3</sup> Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

<sup>4</sup> Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia

<sup>5</sup> Stem Cell and Cancer Institute, Jakarta, Indonesia

## Abstrak

**Latar belakang:** Sel punca mesenkim (MSC) mempunyai kapasitas imunomodulator terhadap penyakit autoimun dan mencegah penolakan imun alogenik. Banyak studi mengungkapkan bahwa MSC menghambat proliferasi sel T dan menginduksi kondisi immunosupresi melalui produksi prostaglandin dan interleukin-10. Pemberian MSC pada pasien autoimun dapat mengurangi autoantibodi di sirkulasi. Sejauh ini belum pernah dilaporkan keberadaan reseptor Fc (reseptor immunoglobulin) pada MSC. Penelitian ini bertujuan untuk mengungkapkan ekspresi reseptor Fc $\gamma$  (Fc $\gamma$ R) pada MSC yang diisolasi dari lipoaspirat dengan mendeteksi mRNA reseptor Fc $\gamma$  dan menganalisis apakah ekspresinya dapat dimodulasi.

**Metode:** MSC hasil isolasi dari lipoaspirat dikultur dalam medium yang sesuai dan dikonfirmasi melalui ekspresi molekul permukaan selnya menggunakan kriteria International Society for Cellular Therapy (ISCT). mRNA total kemudian diisolasi dan dideteksi apakah terdapat ekspresi mRNA Fc $\gamma$ RI, Fc $\gamma$ RIIA dan Fc $\gamma$ RIIB. Ekspresi mRNA tersebut juga dideteksi apakah dapat dimodulasi dengan HAGG dan interferon (IFN) $\gamma$ .

**Hasil:** mRNA reseptor Fc $\gamma$  dapat dideteksi pada MSC pasase pertama. Ekspresi tersebut akan hilang pada pasase sel lebih dari 4. Penambahan IFN $\gamma$  ke dalam kultur menyebabkan peningkatan ekspresi mRNA Fc $\gamma$ RI dan Fc $\gamma$ RIIA. Penemuan ini membuka pandangan baru untuk memahami interaksi dari MSC dan immunoglobulin G melalui reseptor Fc $\gamma$ .

**Kesimpulan:** MSC yang diisolasi dari lipoaspirat mengekspresikan mRNA Fc $\gamma$ R dan ekspresinya dapat dimodulasi dengan IFN $\gamma$ .

## Abstract

**Background:** Mesenchymal stem cell (MSC) has been reported to have immunomodulator capacity against autoimmune diseases and to prevent allogenic tissue rejection. Many studies revealed that MSC's inhibit T cell proliferation and induce immunosuppressive condition through the production of prostaglandins, and interleukin-10. In addition, MSC was reported to reduce circulating autoantibody in autoimmune patients following MSC transfusion. So far, there has been no report stating the presence of Fc receptors (receptors for immunoglobulin) on MSCs. The aim of this study was to reveal the expression of Fc $\gamma$ Rs in lipoaspirate-derived MSCs by measuring transcription of Fc $\gamma$ R mRNA and whether the expression can be modulated.

**Methods:** Lipoaspirate-derived MSCs were cultured in suitable medium and confirmed to be MSCs according to the criteria published by International Society for Cellular Therapy. Total mRNA of MSCs was isolated, and detection of human Fc $\gamma$ RI, Fc $\gamma$ RIIA and Fc $\gamma$ RIIB mRNA was performed. Further, modulation of the expression was tested using heat aggregated gamma globulin (HAGG) and interferon (IFN) $\gamma$ .

**Results:** Fc $\gamma$ Rs mRNA was detected in the first passage of MSCs. However, the expression was no longer present after more than 4 passages. Further, increased level of Fc $\gamma$ RI and Fc $\gamma$ RIIA mRNA expression was detected with the addition of IFN $\gamma$  in the culture. This preliminary finding opens a new insight for the understanding of interaction between MSCs and immunoglobulin G through Fc $\gamma$ Rs.

**Conclusion:** Lipoaspirate-derived MSCs express Fc $\gamma$ Rs, and the expression is modulated by IFN $\gamma$ .

**Keywords:** Fc $\gamma$  receptor, interferon  $\gamma$ , immunoglobulin G, mesenchymal stem cell

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Correspondence author: Dian R. Laksmiawati, dianratih.ffup@gmail.com

Mesenchymal stem cells (MSC) have been shown to have immunosuppressive properties and ability to reduce inflammation.<sup>1</sup> Past studies reported that human MSCs suppressed lymphocyte alloreactivity *in vitro* in mixed lymphocyte cultures (MLC), through human leukocyte antigen (HLA)-independent mechanisms. Moreover, administration of MSCs intravenously, improved some organ injury in animal models through paracrine effects and antiinflammatory cytokines.<sup>1</sup>

Autoantibody in the circulation is mainly immunoglobulin G (IgG), which is responsible for the formation of immune complex. The IgG immune complex may elicit tissue destruction by two mechanisms, either through the activation of complement cascade or activation of inflammatory cells (e.g. macrophages, neutrophils, monocytes, etc.) and platelets following its engagement to IgG receptor namely FcγRs. There are 5 classes of FcγRs, i.e. FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa and FcγRIIIb. Each receptor possesses unique properties along with the different distribution among various inflammatory cell types. To date, there has been no publication reporting FcγRs expression on MSC and the possible downstream cascade that may result following activation of MSC by immune complex. Therefore, it is very interesting to investigate whether MSCs play a potential role in the clearance of immunoglobulin through FcγR mediated immune complex binding.

Mesenchymal stem cells can be isolated from several sources including bone marrow, lipoaspirate, and umbilical cord blood. The plasticity of MSCs isolated from different sources has been reported to be comparable despite some minor differences.<sup>2</sup>

The objective of this study is to detect the expression of FcγRI, FcγRIIa, and FcγRIIb on mesenchymal stem cells isolated from lipoaspirate, and whether the expression can be modulated.

## METHODS

This is an experimental descriptive study, which was done in Stem Cell and Cancer Institute, Jakarta, Indonesia, from May 2010 through May 2011. In this study, cells were isolated from lipoaspirates, cultured, and passaged. Characterization of MSCs was done by flowcytometry using MSC markers. Further, the MSCs were passaged in the presence of either heat aggregated gamma globulin (HAGG)

or interferon gamma (IFN $\gamma$ ) until passage-5. Expressions of FcγRs were determined on passage 1, 2, 4, and 5, and the results were presented in the form of a table.

## MSC isolation from lipoaspirates

Protocols used in this study have been reviewed and approved by the Stem Cell and Cancer Institute Institutional Review Board prior to the study (number 19/IRB/SCI/KF/2010). Lipoaspirates, which were discarded waste materials from individuals who experienced tumescent liposuction were obtained from 3 hospitals in Jakarta. Lipoaspirates were stored at 2–8°C for no longer than 24 hours before they were used. The methods used to isolate the mesenchymal stem cells from lipoaspirate were adapted from the methods in Zuk, et al<sup>3</sup> and Sardjono, et al<sup>4</sup>

## Flowcytometry assay to characterize MSC population

To confirm the characteristic of MSCs isolated, several assays were conducted. We use positive (CD105, CD73) and negative MSCs markers (HLA-DR, CD 14, CD 19, CD 45 and CD 34) according to the ISCT minimal criteria for defining MSC.<sup>5</sup> Cells were harvested and stained with the appropriate surface monoclonal antibodies (PE conjugated: CD105 (abcam 53321-100), CD73 (BD550257), HLA-DR (abcam 23901), CD 19 (abcam 1168-500); FITC-conjugated CD14 (abcam 28061-100), CD45 (BD 555482) and CD34 (BD 348053) following manufacturer's instructions. Isotype controls i.e. mIgG1 (BD349041 and BD 349043), and mIgG2 (BD349053) were used to determine background staining. After washing, the cells were fixed, and 5000 events were acquired using a FACSCalibur 3 argon laser 488nm (Becton Dickinson) for each marker. Cell-Quest-pro software was used for acquisition and analysis of the percentage of marker bearing MSCs.

## HAGG preparation

Heat aggregated gamma globulin was prepared from 8-10 mg/mL of human gamma globulin (Gammaraas immune globulin IV 5%, Shanghai RAAS) heated for 30 minutes at 63°C, followed by adjustment of the concentration to 1% (w/v) with polyethylene glycol 6000 (Sigma) in PBS and kept on ice for 30 minutes. The precipitated complexes

were centrifuged (10.000 g at 4°C for 10 minutes), the supernatant was discarded and complexes were dissolved in PBS at 1 mg/mL.

### Stimulation of MSC cultures

When passage 1 of MSC culture reached 80% confluence, cells was harvested and seeded in 3 culture discs. Cells in two discs were stimulated by addition of either HAGG or hIFN $\gamma$  (Roche 11040596001). Cultures were maintained until the cells reached 80% confluence. Culture media were changed every 3-4 days.

### Detection of Fc $\gamma$ Rs mRNA expression

Whole mRNA were isolated from 10<sup>6</sup> MSC cells and K562 cell line as positive control, using GenElute™ Direct mRNA MiniPrep kit (Sigma, DMN10). The cDNA were made using Transcriptor First Strand cDNA synthesis Kit (Roche, 04 379 012 001) and PCR to amplify Fc $\gamma$ Rs mRNA was performed using oligonucleotides specific for human Fc $\gamma$ Rs (Table 1).

The amount of PCR-amplified products were visualized by ethidium bromide and semi-quantified as per beta-actin mRNA expression.

## RESULTS

Consistent with the definition of criteria for MSCs by International Society of Celluler Therapy (ISCT), the cultured MSCs in this study exhibited the fibroblast-like characteristic,<sup>5</sup> (Figure 1). To confirm whether the isolated cells are mesenchymal stem cells, we characterized some surface molecules according to ISCT. By the flowcytometry assay we found that the cell do not express hematopoietic or endothelial surface marker CD14, CD19, CD34 CD45 and HLA-DR but stain positive for CD105 and CD73 (more than 90%). The surface markers of MSCs at passage-2 can be seen in figure 2.

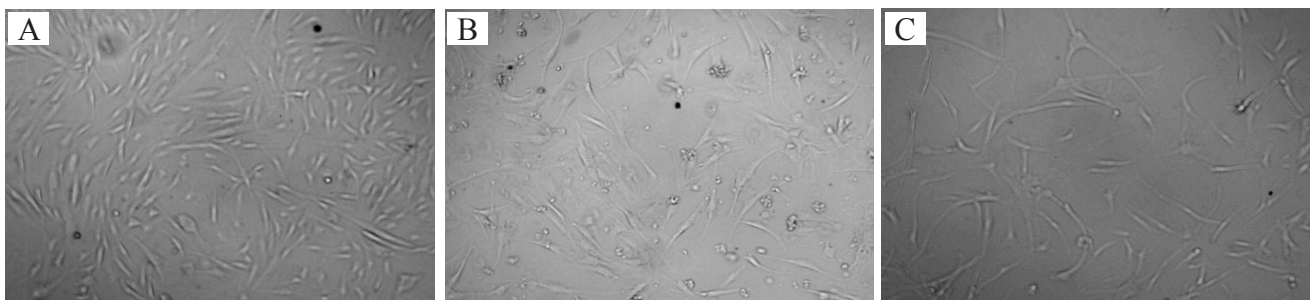
Further, mRNA expressions of Fc $\gamma$ RI, Fc $\gamma$ RIIA, Fc $\gamma$ RIIB in K562 cell line (positive control) and MSCs passage 1 up to 5, which were cultured with and without HAGG or IFN $\gamma$  can be seen in figure 3.

## DISCUSSION

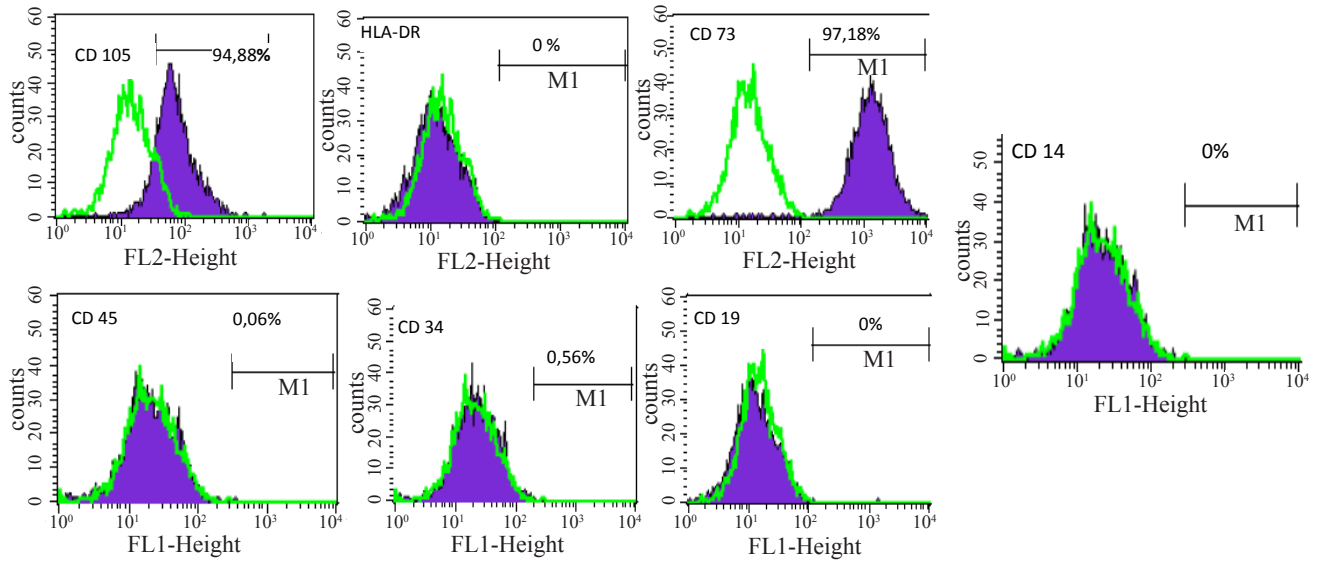
Because of immunosuppressive property of MSCs, nowadays these cells were used for the therapy of graft vs host disease (GvHD) and autoimmune disease. Some researchers studied the mechanism

**Table 1.** Sequence of oligonucleotides and product size

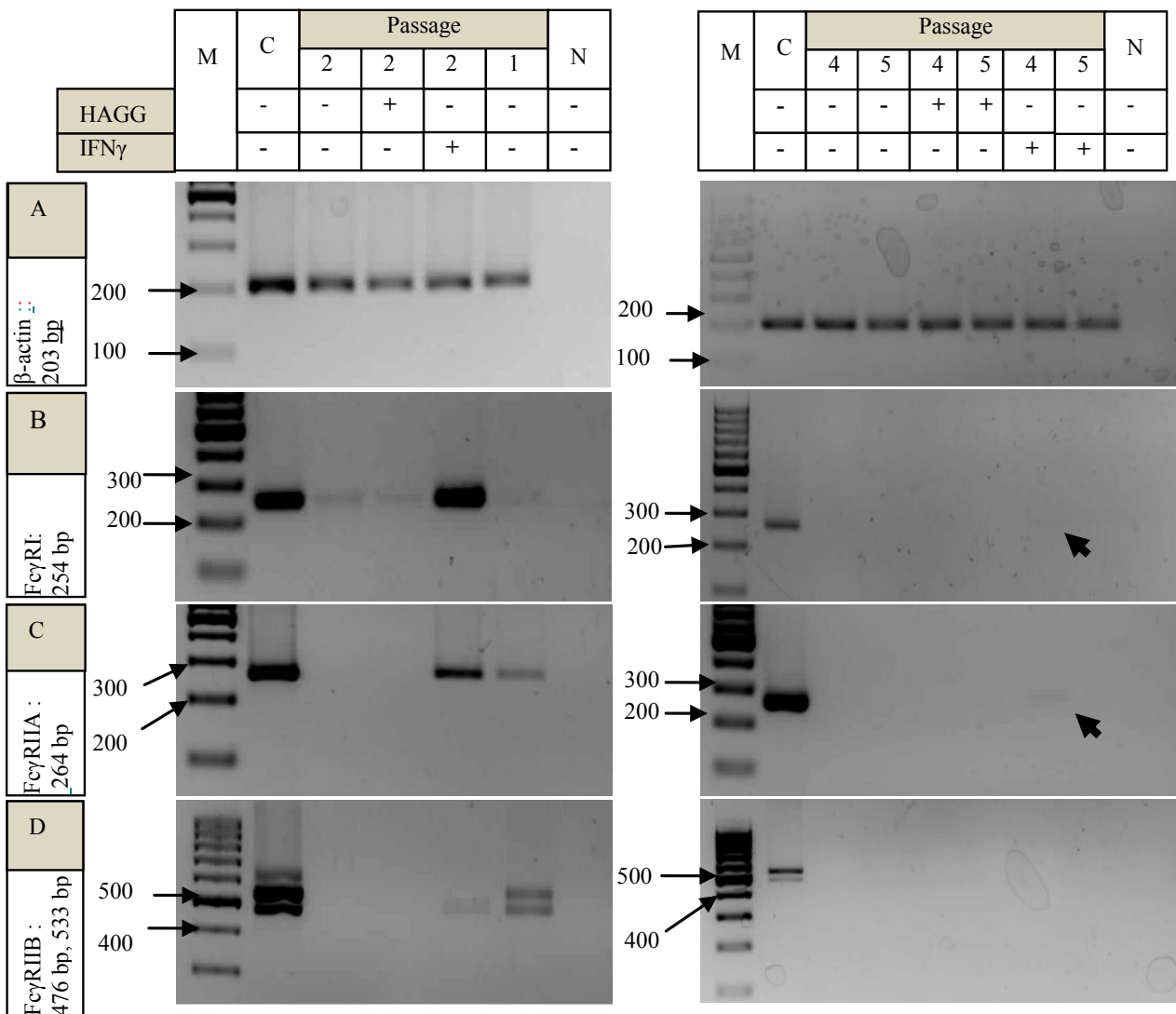
Oligo-nucleotides	Sequences (5' → 3')	Product size (bp)
Fc $\gamma$ R I	5'- GCTCCAGTGCTGAATGCATC -3' 5'- ACTCAGGGCTGCGCTTAAGG -3'	255
Fc $\gamma$ R IIA	5'-TTCCACACCCCTTCCACTCTG-3' 5'-AACAGATTCATCCGTCCTGGC-3'	265
Fc $\gamma$ R IIB	5'-CCTCACCTGGAGTTCAGGAGGGAG-3' 5'-AGACAATGGAGACTAAATACG-3'	534 and 477
$\beta$ -Actin	5'-CCTCGCCTTTGCCGATCC-3' 5'-GGAATCCTTCTGACCCATGC-3'	205 bp



**Figure 1.** Morphology of MSC from lipoaspirate at day 4th, showing fibroblasts-like cell. A: untreated MSC, B : HAGG-stimulated MSC, C:IFN $\gamma$ -stimulated MSC



**Figure 2.** Flowcytometry analysis of surface molecules on MSCs from lipoaspirate. M: marker (MSCs in the population that positive bearing certain marker). This data was acquired at MSC in passage-2. ■ : culture cell ■ : isotype control



**Figure 3.** mRNA expressions of Fc $\gamma$ RI, Fc $\gamma$ RIIA, Fc $\gamma$ RIIB. M: marker, C: K562 cell line, as positive control, N : No template control. MSCs, passage -1 to -5, were cultured with/ without HAGG or IFN $\gamma$ . Bold arrows show faint bands

of immunosuppressive property of MSCs and found that there were some substances that were secreted by MSCs such as TGF- $\beta$ 1, IL-10, IL6, prostaglandin E2, nitric oxide and indoleamine 2,3-dioxygenase (IDO).<sup>6-10</sup>

Until now, there was no report about the presence of Fc $\gamma$ Rs on MSCs. Fc $\gamma$ Rs are the receptors of an Fc arm of immunoglobulin G, which links humoral and cellular immune function. This study was the first report as preliminary finding that opens a new insight for the understanding of interaction between MSCs and immunoglobulin G through Fc $\gamma$ Rs.

In unstimulated cultures, we found that there were mRNAs of Fc $\gamma$ RI, Fc $\gamma$ RIIA and Fc $\gamma$ RIIB but they were only observed at the passage-1, except for Fc $\gamma$ RI that was still observed at passage-2 (Figure 3). The factor that might be the reason was that in fact, MSCs are actually present as myriads of MSCs at different stages of differentiation, which was proven in our study that showed the various percentages of the markers, though their morphology was still fibroblastic. Certain MSCs might bear the Fc $\gamma$ Rs, while others might not. The proportion of Fc $\gamma$ Rs bearing MSCs might decrease after passages.

Effort to stimulate Fc $\gamma$ R was done with IFN $\gamma$  addition to passage-2 of MSC culture. The result showed that expected band of mRNAs of Fc $\gamma$ RI, Fc $\gamma$ RIIA and Fc $\gamma$ RIIB1 were still observed at passage-2, even though band of Fc $\gamma$ RI and Fc $\gamma$ RIIA mRNA were observed faintly. Result showed that Fc $\gamma$ RI, Fc $\gamma$ RIIA and Fc $\gamma$ RIIB1 bands were still observed at passage-2 of IFN $\gamma$ -stimulated MSCs. The bands of Fc $\gamma$ RI and Fc $\gamma$ RIIB1 were still faintly detected in passage-4 of IFN $\gamma$ -stimulated MSCs. Further, the addition of HAGG did not stimulate Fc $\gamma$ RI, Fc $\gamma$ RIIA and Fc $\gamma$ RIIB in mRNA level (Figure 3).

In a study by Yi Li, 2010, there was an increase in Fc $\gamma$ RI expression on systemic lupus erythematosus compared to normal individuals. Apparently, the fact might be due to the increase in IFN $\gamma$  that was found in SLE patient.<sup>11</sup> The effect of IFN $\gamma$  on the expression of Fc $\gamma$ RI and Fc $\gamma$ RIIB mRNA level was also studied by Quan, et al<sup>12</sup> on microglia. Using quantitative real-time PCR they showed that IFN- $\gamma$  induced a 4-fold increase in the mRNA level of Fc $\gamma$ RI, but did not induce changes in Fc $\gamma$ RIIB expression.<sup>12</sup>

In conclusion, this study showed that Fc $\gamma$ Rs mRNAs were detected at passage-1 of MSCs, whilst on the subsequent passages (after 4 passages) the expressions of Fc $\gamma$ Rs mRNAs were no longer observed. Further, the expression was modulated by IFN $\gamma$ .

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### Conflict of interest

All authors have nothing to disclose.

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