Acta Paediatrica Hungarica 29 (1–2) pp. 37–40 (1988–89)

Ligation of FcRI and FcRII of human mononuclear phagocytes

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Recently, increasing evidence has indicated a molecular and functional heterogeneity of Fc receptor for IgG (FcR) on human monocytes. The first, FcRI, binds monomeric IgG1 and red cells coated by human anti-Rh IgG (1). Most recently, Dougherty et al. [4] published that erythrocytes opsonized with rabbit IgG (EA) bind also to monocyte FcRI. A monoclonal antibody (mAb) designated 10.1 by Dougherty et al. inhibited the binding of EA to FcRI.

In this study we used monoclonal antibodies against FcRI designated 32, and against FcRII designated IV3, which were generous gifts of C. L. Anderson, for inhibition of EA binding and ingestion. In addition, the effect of anti-FcRI and anti-FcRII on superoxide anion generation of monocytes were investigated.

Results and Discussion

Sheep red blood cells (SRBC) sensitized with specific rabbit IgG bind to monocytes via their FcR at 4 °C for 60 min. When the rabbit anti-SRBC IgG was diluted 1: 128 70–80% of the monocytes form stable rosettes (Fig. 1). Preincubation of monocytes with anti-FcRI for 30 min at 4 °C resulted in a marked inhibition of EA binding. Pretreatment of monocytes with anti-FcRII caused a moderate inhibition in the rosette formation. Control murine IgG1 and IgG2b had no inhibitory effect. These data suggest that anti-FcRI interferes with the high affinity sites of FcR responsible for EA binding on human monocytes.

SRBC sensitized with specific rabbit IgG were ingested by monocytes at 37 °C for 20 min (Fig. 2). Preincubation of monocytes with anti-FcRII inhibited the EA ingestion in a dose-dependent extent. In addition, pretreatment of monocytes with anti-FcRI caused only a slight inhibition in the EA ingestion. Control murine IgG1 and IgG2b had no inhibitory effect. We can conclude from these data that anti-FcRII interferes with an epitope of FcRII which may be involved in the mechanism of endocytosis i.e. the ingestion of the bound EA.

It is known that antibody against FcRII designated IV3 has only a partial effect on the binding of EA by monocytes [1]. Our present results

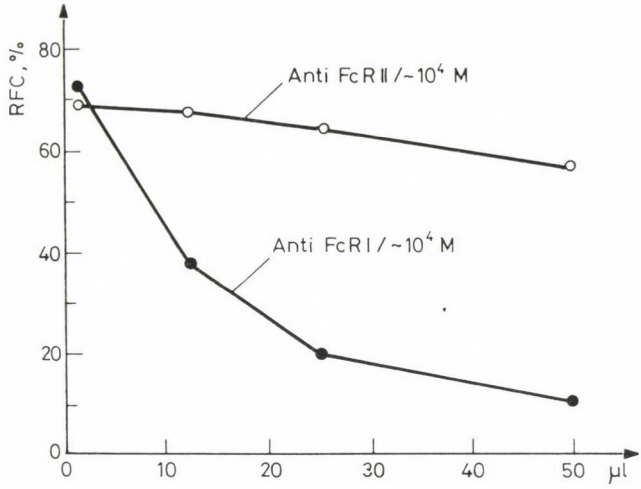

FIG. 1. *The inhibition (30 min at 4 °C) of EA binding (60 min at 4 °C) to monocytes with antibodies to FcRI and FcRII* Human mononuclear blood cells were purified by adherence to glass surface (5). The monocyte (M) monolayers ($1-2\times10^4$ cells contained more than 80% monocytes) were preincubated in Parker 199 medium, pH 7.4, without and with 12.5—50 µl of anti-FcRI and anti-FcRII (1—10 µg/ml) at 4 °C for 30 min then they were incubated with 100 µl of 2.5% red cells sensitized with rabbit IgG (EA) at 4 °C for 60 min. After washing, the cells were staining with 0.2% crystal violet and the percentage of the rosette forming cells (RFC) was determined

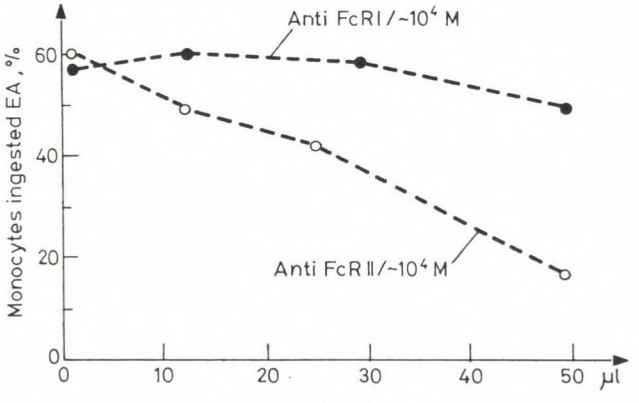

FIG. 2. *The inhibition (10 min at 20 °C) of EA ingestion (20 min at 37 °C) by monocytes with antibodies to FcRI and FcRII* The monocyte (M) monolayers were preincubated at 22 °C for 10 min without and with 12.5—50 µl of anti-FcRI and anti-FcRII in Parker medium, then they were incubated with 100 µl of 2.5% EA at 37 °C for 20 min. After washing, the EAs bound to the cell surface were lysed by water for 30 sec, then the monolayers were washed with Parker medium and stained with 0.2% crystal violet. The percentage of monocytes ingested 3 or more EA was determined

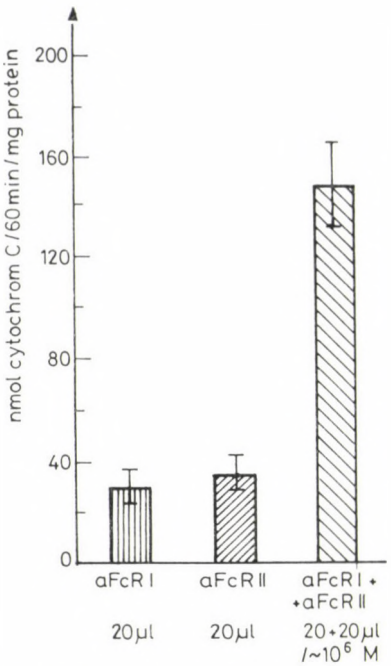

FIG. 3. *Increased production of O_2^- by monocytes stimulated simultaneously with anti-FcRI and anti-FcRII* The mononuclear cells contained approximately 10^6 monocytes (M) in 25 mM Hepes-buffered Hanks' balanced salt solution, pH 7.4, were incubated with 80 µM ferricytochrome C and 1000 U/ml catalase as well as without and with anti-FcRI and anti-FcRII or simultaneously with the two antibodies. Superoxide dismutase was added to duplicate control tubes to 90 µg/ml. Superoxide radicals were expressed as nmol of ferricytochrome C reduced per mg protein per 60 min (3)

strongly suggest that the particulated IgG complexes bound by the FcRI move to FcRII for endocytosis which is preceded by either patching or capping of the complex [6].

Anti-FcRI and anti-FcRII generated superoxide anion which was inhibitable by superoxide dismutase (Fig. 3). The ligation of the antibody with specific receptor initiates the signal transduction mechanism, which leads to the secretion of reactive oxigen intermediers (ROI). When the monocytes were stimulated simultaneously with anti-FcRI and anti-FcRII the ROI secretion was not only superposed but amplified. As positive control, the monocytes were stimulated with rabbit IgG-coated latex and as negative control with murine IgG1 or IgG2b.

In our experiment each of the two anti-FcR mAbs stimulated the release of ROI from monocytes. However, incubation of U937 cells with mAb32 and IV3 alone or cross-linked did not result in superoxide anion generation [2].

Taken as whole, our present results strongly suggest that it can be some cooperation between the signal transduction and the ligations of FcRI and FcRII.

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