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Different abilities of two distinct Fcγ receptors on guinea pig polymorphonuclear leukocytes to trigger the arachidonic acid metabolic cascade

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The nature of signals transmitted by two Fcγ receptors ($Fc\gamma_2R$ for IgG2 and $Fc\gamma_1/\gamma_2R$ for both IgG2 and IgG1) on guinea pig polymorphonuclear leukocytes was investigated. The specific binding of hen ovalbumin (OA)-complexed IgG1 antibody to $Fc\gamma_1/\gamma_2R$ did not seem to trigger the release of [^{3}H]arachidonic acid from the cells prelabeled with [^{3}H]arachidonic acid. In contrast, marked release occurred on the binding of OA-complexed IgG2 antibody to the cells. This reaction was not inhibited, but rather enhanced, by the Fab' of a monoclonal antibody to $Fc\gamma_1/\gamma_2R$. Therefore, a signal for the activation of the arachidonic acid metabolic cascade was found to be transmitted by $Fc\gamma_2R$, but scarcely by $Fc\gamma_1/\gamma_2R$.

Arachidonic acid; Fcγ receptor; Anti-Fcγ receptor antibody; Immune complex; Polymorphonuclear leukocyte

1. INTRODUCTION

The Fcγ receptors are the surface proteins of phagocytes which bind specifically to the Fc portions of antigen-complexed IgG antibodies, resulting in the elicitation of various functions of the cell such as the respiratory burst, phagocytosis and activation of the arachidonic acid metabolic cascade [1-4]. Recently, the variety of FcγRs on a single phagocytic cell was made clear with human and murine cells [5]. We also demonstrated that guinea pig polymorphonuclear leukocytes express two distinct types of FcγR; one ($Fc\gamma_2R$) is specific for IgG2 alone and the other ($Fc\gamma_1/\gamma_2R$) is specific for both IgG1 and IgG2 [6].

Recently, we found that a signal for the activation of the arachidonic acid metabolic cascade was transmitted by $Fc\gamma_2R$, but scarcely by $Fc\gamma_1/\gamma_2R$. This finding is reported in this paper.

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Abbreviations: PMN, guinea pig polymorphonuclear leukocytes; FcγR, Fcγ receptor; $Fc\gamma_2R$, Fcγ receptor for IgG2; $Fc\gamma_1/\gamma_2R$, Fcγ receptor for IgG1 and IgG2; OA, hen ovalbumin; KRPG, Krebs Ringer phosphate buffer supplemented with 5 mM D-glucose, pH 7.4; OA-IgG1, ovalbumin-complexed IgG1 antibody; OA-IgG2, ovalbumin-complexed IgG2 antibody

2. MATERIALS AND METHODS

2.1. *Materials*

Guinea pig PMNs were isolated 15 h after intraperitoneal injection of casein, and purified as described in [7]. Guinea pig IgG1 and IgG2 anti-OA antibodies were highly purified from hyperimmune serum, as described in [8]. The monoclonal antibody (VIA2) IgG1) to guinea pig macrophage $Fc\gamma_1/\gamma_2R$ used was a secreting product of the hybridoma cells (VIA2) obtained by cell fusion of the splenic cells of a mouse immunized with

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guinea-pig intraperitoneal macrophages with murine myeloma cells. The antibody was highly purified and its Fab' was prepared as described in [9].

2.2. *Measurement of the release of [^{3}H]arachidonic acid from the prelabeled PMNs*

PMNs (2.5×10^8 cells) were incubated with 10 μCi of [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid (New England Nuclear, Boston, MA) for 15 min at 37°C in 25 ml of Eagles minimum essential medium supplemented with 20 mM Hepes and 0.1% bovine serum albumin, pH 7.4. Following washing with KRPG, the cells were suspended in KRPG at 3×10^7 cells/ml.

The PMNs thus prelabeled (6×10^6 cells) were stimulated with OA-complexed IgG1 or IgG2 antibody (OA-IgG1 or OA-IgG2) in 1 ml of KRPG for 15 min at 37°C, and then centrifuged. The amount of [^{3}H]arachidonic acid released from the cells was determined by measuring radioactivity of the supernatant in a scintillation counter. These procedures were essentially the same as those described by Hirata et al. [10]. As a control, PMNs were similarly incubated, but without OA-IgG. The amount of [^{3}H]arachidonic acid released was expressed as a percentage of that of the cells used, and the results were shown by the release enhanced by OA-IgG; the spontaneous release was about 1% in most of the experiments.

3. RESULTS

3.1. *The release of arachidonic acid with antigen-complexed IgG antibodies*

Phagocytes including PMNs release arachidonic acid on stimulation with immune complexes [3,4]. To investigate the possible roles of Fcγ_2R and Fcγ_1/γ_2R on guinea pig PMNs in the reaction, the cells were stimulated with OA-IgG2 and OA-IgG1 which had been prepared by reacting each antibody with the antigen at a molar antigen to antibody ratio of 0.1. The stimulation with OA-IgG2 elicited a marked release of [^{3}H]arachidonic acid from the prelabeled cells in a time-dependent manner (fig.1). This ability of OA-IgG2 was of the same order of magnitude as that of 1 μM ionomycin which is a Ca^{2+}-specific ionophore [11]; at this concentration, ionomycin exhibited the highest activity. The amount of [^{3}H]arachidonic acid released for 15 min increased with increasing OA-IgG2 up to 200 μg; the amount of complex was expressed in terms of IgG2 antibody used for preparing the complex (fig.2).

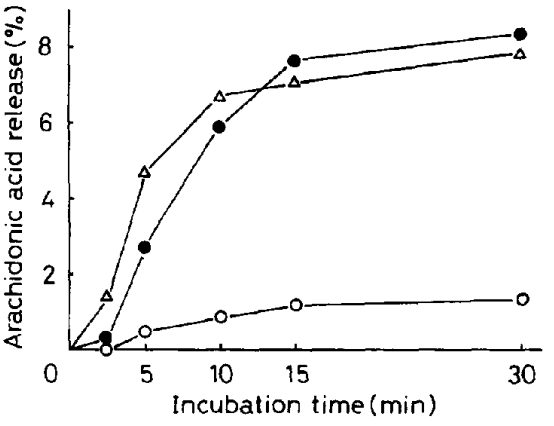

Fig.1. Time courses of the arachidonic acid release by PMNs stimulated with OA-IgG1, OA-IgG2 and ionomycin. [^{3}H]Arachidonic acid-prelabeled PMNs (6×10^6 cells) were incubated with 200 μg/ml of OA-IgG1 (○), OA-IgG2 (●) or 1 μM ionomycin (△) for varying periods at 37°C. After incubation, the amounts of released [^{3}H]arachidonic acid were measured. Each value expresses the total amount of released [^{3}H]arachidonic acid without subtracting the spontaneous release.

In contrast, the ability of OA-IgG1 to elicit the release of [^{3}H]arachidonic acid was markedly low even when the cells were reacted with excessive amounts of the complex (figs 1 and 2). Though the ability of OA-IgG1 was further tested with the complexes prepared by reacting 200 μg IgG1 antibody with OA at varying molar antigen to antibody ratios from 0.025 to 0.5, the amounts of released [^{3}H]arachidonic acid were remarkably small, relative to those with OA-IgG2. These results indicate that the Fcγ_1/γ_2R-mediated stimulation of PMNs scarcely induces any intracellular event(s) leading to the activation of the arachidonic acid metabolic cascade. On the other hand, the highest activity of OA-IgG2 was shown by the complexes formed at molar antigen to antibody ratios of 0.1–0.5 (not shown).

The release of [^{3}H]arachidonic acid by the OA-IgG2-stimulated PMNs was completely inhibited either by 5 μM *p*-bromophenacyl bromide (a

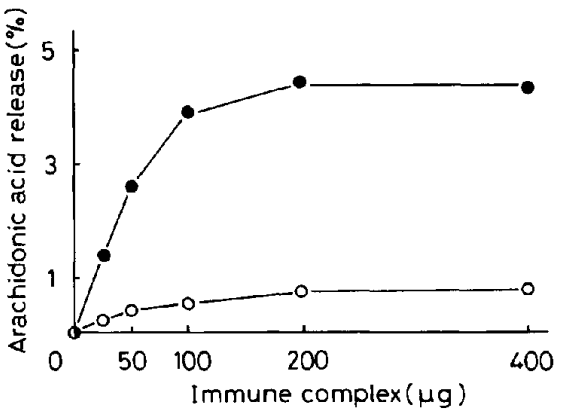

Fig.2. The arachidonic acid release by PMNs stimulated with OA-IgG1 and OA-IgG2. [^{3}H]Arachidonic acid-prelabeled PMNs (6×10^6 cells) were incubated with varying amounts of OA-IgG1 (○) or OA-IgG2 (●) for 15 min at 37°C. After incubation, the amounts of released [^{3}H]arachidonic acid were measured.

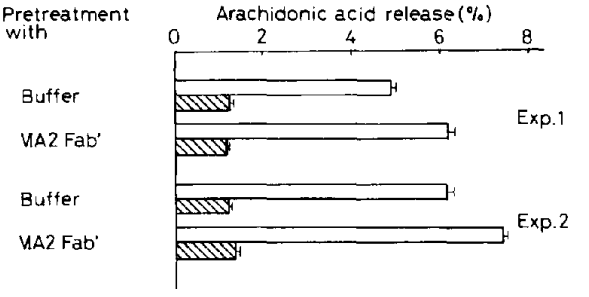

Fig.3. Effect of the Fab' of anti-Fcγ_1/γ_2R antibody on the arachidonic acid release by OA-IgG2-stimulated PMNs. [^{3}H]Arachidonic acid-prelabeled PMNs (6×10^6 cells) were incubated with or without 10 μg of the Fab' of anti-Fcγ_1/γ_2R antibody (VIA2 Fab') for 1 h at 4°C, and further incubated in the presence (open bars) or absence (hatched bars) of 200 μg of OA-IgG2 for 15 min at 37°C. After incubation, the amounts of released [^{3}H]arachidonic acid were measured. Each value represents the mean ± SD.

phospholipase A_2 inhibitor) or by 0.5 mM EGTA (not shown). Thus, arachidonic acid release by the stimulation with OA-IgG2 may be a direct result of the initial activation of intrinsic phospholipase A_2 activity. On the other hand, 0.5 μg/ml of cytochalasin B did not change the levels of [^{3}H]arachidonic acid release from the OA-IgG2- and OA-IgG1-stimulated PMNs (not shown). Since cytochalasin B inhibits phagocytic activity [12], the results obtained on arachidonic acid release may be independent of the phagocytic process.

3.2. *The effect of anti-Fcγ_1/γ_2R antibody on the reaction with OA-IgG2*

The results described in the preceding section strongly suggest that the triggering signal for the arachidonic acid release is transmitted by Fcγ_2R, but scarcely by Fcγ_1/γ_2R. To confirm further this possibility, the effect of a monoclonal antibody to macrophage Fcγ_1/γ_2R. (VIA2 IgG1) on the release of [^{3}H]arachidonic acid with OA-IgG2 was examined because the antibody binds cross-reactively to the Fcγ_1/γ_2R on PMN cells [6].

When the prelabeled PMNs were preincubated with the Fab' of VIA2 IgG1 and then reacted with OA-IgG2, the release of [^{3}H]arachidonic acid was not inhibited, but rather enhanced 22–36% (fig.3). This suggests that the release of [^{3}H]arachidonic acid with OA-IgG2 is mediated by Fcγ_2R alone, and also that the binding of OA-IgG2 through Fcγ_1/γ_2R, which occurs concurrently with the binding through Fcγ_2R, lowers competitively the ability of Fcγ_2R to elicit the arachidonic acid release.

4. DISCUSSION

The results presented in this paper demonstrate that two types of FcγR on the surface of PMN cells are distinguishable from each other with regard to function. The specific binding of OA-IgG2 to Fcγ_2R activated the intrinsic phospholipase A_2 activity, leading to the enhanced release of arachidonic acid. On the other hand, the specific binding of OA-IgG1 or OA-IgG2 to Fcγ_1/γ_2R did not significantly trigger any enhanced release of arachidonic acid. As the number of Fcγ_1/γ_2R is 2×10^4 molecules per PMN cell, which is about one-quarter of that of Fcγ_2R molecules [6], the failure of OA-IgG to trigger arachidonic acid release through Fcγ_1/γ_2R might be due to the small number of Fcγ_1/γ_2R molecules per cell. However, the inhibition of the Fcγ_1/γ_2R-mediated binding of OA-IgG2 by the Fab' of anti-Fcγ_1/γ_2R antibody resulted in the enhancement of arachidonic acid release with the complex, indicating that Fcγ_1/γ_2R

does not cooperate with Fcγ_2R in the reaction, but depresses the Fcγ_2R-mediated reaction.

We, furthermore, confirmed that the stimulation of PMNs with OA-IgG1 could elicit both the O_2^--generating and phagocytic activities, as in the case of OA-IgG2 (data to be published elsewhere). It, therefore, seems very probable that in differing from Fcγ_2R, Fcγ_1/γ_2R cannot transmit sufficient signal for the activation of phospholipase A_2 activity, and that these two FcγRs transmit two unrelated, distinct signals to the cells, upon specific binding of appropriate immune complexes.

Recently, a signal for the activation of the arachidonic acid metabolic cascade was reported to be transmitted by Fcγ_{2b}R, but not by Fcγ_{2b}R, on the surface of a murine macrophage cell line, P388D$_1$ cells [4]. In this respect, the Fcγ_2R and Fcγ_1/γ_2R that we studied resemble Fcγ_{2b}R and Fcγ_{2a}R on P388D$_1$ cells, respectively.

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