Vol. 172, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1990 Pages 223-228

TWO DISTINCT TYPES OF Fc RECEPTOR FOR IgG ON GUINEA PIG MACROPHAGES ACTIVATE THE NADPH OXIDASE THROUGH DIFFERENT SIGNAL-TRANSDUCTION PATHWAYS

Tomozumi Imamichi and Jiro Koyama*

Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-ku, Sapporo 060, Japan

Received August 30, 1990

Neither 5 μM calmodulin antagonist W-7 nor depletion of the intracellular Ca^{2+} affected the O_2^- generation triggered by the Fc receptor for IgG2 (Fcγ_2R) on guinea pig macrophages, although that by the Fc receptor for both IgG1 and IgG2 (Fcγ_1/γ_2R) was lowered to 50% of a control level by W-7 and the Ca^{2+} depletion, respectively. Staurosporine inhibited all these responses, but to different extents. These results reveal that the signal-transduction pathways linked to these receptors differ from each other, and that Fcγ_1/γ_2R triggers the O_2^- generation through the Ca^{2+}-calmodulin system and the Ca^{2+}-independent system.

Guinea pig macrophages possess two distinct types of Fc receptor for IgG (FcγR); one is specific for IgG2 alone (Fcγ_2R) and the other binds both IgG1 and IgG2 (Fcγ_1/γ_2R) (1,2). Recently, a cDNA coding for Fcγ1/γ2R was cloned, showing that the FcγR structually belongs to the group 2 FcγRs (mouse FcγR II$_{B2}$ and human FcγR II) (3). These two FcγRs trigger the O_2^--generating response on binding of antigen-complexes of IgG1 and IgG2 antibodies. In a previous paper (4), we reported that the O_2^- generation triggered by Fcγ_1/γ_2R was lowered to 50% of a control level by depletion of the intracellular Ca^{2+} by incubating the cells with Ionomycin and EGTA, although the Ca^{2+} depletion did not affect the O_2^- generation by Fcγ_2R. These results suggest strongly that the Fcγ_2R- and Fcγ_1/γ_2R- mediated signal-transduction processes for the activation of the NADPH oxidase differ from each other.

We, recently, found that the O_2^- generation triggered by Fcγ_1/γ_2R was 50% inhibited with N-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide

*To whom correspondence should be addressed.

Abbreviations: FcγR, Fc receptor for IgG; Fcγ_1/γ_2R, Fc receptor for IgG1 and IgG2; Fcγ_2R, Fc receptor for IgG2; KRPB-G, Krebs-Ringer phosphate-buffered saline supplemented with 5.5 mM D-glucose, pH 7.4; OA, ovalbumin; OAγ_1, ovalbumin complex of IgG1 antibody; OAγ_2, ovalbumin complex of IgG2 antibody; PMA, phorbol myristate acetate; SOD, superoxide dismutase: W-7, N-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide .

0006-291X/90 $1.50

(calmodulin antagonist W-7). In contrast, both the O_2^- generation triggered by Fcγ_2R and the Ca^{2+}-independent O_2^- generation by Fcγ_1/γ_2R were not inhibited. In addition, all these responses were found to be inhibited with staurosporine, but to different extents. In this paper, these results are reported, indicating that the signal-transduction pathways linked to these FcγRs differ from each other.

MATERIALS AND METHODS

Materials: Peritoneal macrophages were isolated from Hartley guinea pigs injected intraperitoneally with liquid paraffin. The peritoneal exudate cells were cultured in glass dishes, and the adherent cells were collected and used as macrophages (5). Ca^{2+}-depleted macrophages were prepared by incubation with 1 μM Ionomycin and 0.5 mM EGTA in Ca,Mg-free Krebs-Ringer phosphate-buffered saline supplemented with 5.5 mM D-glucose, pH 7.4 (KRPB-G) for 10 min at 37℃ (4,6). After washing with Ca,Mg-free KRPB-G, the cells were suspended in the same medium. This treatment of macrophages caused a marked decrease in the intracellular concentration of Ca^{2+} from 218 ± 24 to 54 ± 2 nM, when estimated by the use of Fura-2 (4,7).

Guinea pig IgG1 and IgG2 antibodies to ovalbumin (OA) were specifically purified (4,8), and the OA complexes used were prepared by incubating the antibodies with OA at a molar antigen:antibody ratio of 0.1. Monoclonal anti-Fcγ_1/γ_2R antibody (VIA2 IgG1) was purified from ascites fluid of BALB/c mice injected intraperitoneally with hybridoma (VIA2) secreting the antibody. The Fab' of the antibody was prepared, as described in (1).

The following materials were obtained from the indicated sources: ferricytochrome c and superoxide dismutase (SOD) from Sigma Chemical Co., St Louis, MO; Ionomycin from Calbiochem-Behringer, La Jolla, CA; phorbol myristate acetate (PMA) from Seikagaku Kogyo Co., Tokyo, Japan; W-7 from Kyowa Hakko Kogyo Co., Tokyo, Japan.

Measurement of the O_2^-- generating responses: The O_2^--generating response triggered by Fcγ_2R was measured by stimulation of anti-Fcγ_1/γ_2R Fab'-treated macrophages with OA complex of IgG2 antibody (OAγ_2) (4). For this purpose, macrophages (1 X 10^6 cells) were pre-incubated with 100 μg of anti-Fcγ_1/γ_2R Fab' in 0.3 ml of KRPB-G for 1 hr at 4℃, and then stimulated with OAγ_2. The O_2^--generating response triggered by Fcγ_1/γ_2R was measured by stimulation of untreated macrophages with OAγ_1 (4). These O_2^--generating responses were determined by measuring the SOD-inhibitable reduction of ferricytochrome c in a Hitachi double beam recording spectrophotometer U-3200 (9). Sample and reference cuvettes contained anti-Fcγ_1/γ_2R Fab'-treated or untreated macrophages (1 X 10^6 cells) and 50 nmol of ferricytochrome c in 0.95 ml of KRPB-G. The reference cuvette also contained 50 μg of SOD. The reaction was initiated by addition of 100 μg of OAγ_2 or OAγ_1 in 0.05 ml of KRPB-G at 37℃. The difference spectrum was monitored at 550 nm, and the O_2^--generating response was estimated by measuring an initial velocity of the SOD-inhibitable reduction of ferricytochrome c. When the O_2^- generation by Ca^{2+}-depleted macrophages was measured, KRPB-G was replaced by Ca,Mg-free KRPB-G (4). The O_2^--generating response induced by PMA was determined by addition of PMA at a final concentration of 100 ng/ml; the procedures used for this purpose were the same as those described above.

RESULTS AND DISCUSSION

In a previous paper (4), we reported that the O_2^--generating response triggered by Fcγ_1/γ_2R in guinea pig macrophages was lowered to 50% of a control level by depletion of the intracellular Ca^{2+} by incubating the cells with Ionomycin and EGTA, differing from its Fcγ_2R counterpart.

Furthermore, the addition of Ca^{2+} to the Ca^{2}-depleted cells was found to recover completely the O_2^--generating response triggered by $Fc\gamma_1/\gamma_2R$. These findings suggest strongly that the triggering mechanisms of $Fc\gamma_1/\gamma_2R$ and $Fc\gamma_2R$ leading to the activation of the NADPH oxidase differ from each other, and also that $Fc\gamma_1/\gamma_2R$ triggers the activation of the NADPH oxidase through two different signal-transduction processes which differ from each other in dependence upon the intracellular Ca^{2+}.

To elucidate further these different signal-transduction processes, the effect of calmodulin antagonist W-7 (10-12) on the O_2^- generation by stimulated macrophages was determined. When macrophages were stimulated with $OA\gamma_1$, $OA\gamma_2$ or PMA, 100 μM W-7 abolished the O_2^--generating responses. This effect of W-7 may be caused by inhibition of protein kinase C (13,14). However, it seems partly due to the cytotoxic action of W-7, since the same concentration of W-7 lowered the cell viability to 50%, when estimated by a trypan blue exclusion test (data not shown). A lower concentration of W-7 (5 μM), on the other hand, was found to inhibit by 50% the O_2^- generation triggered by $Fc\gamma_1/\gamma_2R$ without any effect on the cell viability, although it exhibited no inhibitory effect on the O_2^- generation by macrophages stimulated through $Fc\gamma_2R$ or with PMA (Fig.1). Furthermore, the O_2^- generation by Ca^{2+}-depleted macrophages stimulated through $Fc\gamma_1/\gamma_2R$ was also not inhibited at all with 5 μM W-7. These results reveal that the O_2^--generating response triggered by $Fc\gamma_1/\gamma_2R$ proceeds through two different signal-transduction systems: the Ca^{2+}- calmodulin system and the Ca^{2+}-independent system. In contrast, that by $Fc\gamma_2R$ seems to proceed through the Ca^{2+}-independent system alone.

Staurosporine (15) is a powerful inhibitor of the respiratory burst induced by various stimulants (16-18). It, however, does not alter the O_2^- generation by cell-free preparations of the NADPH oxidase (18). We,

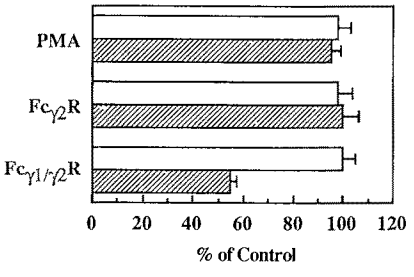

Fig.1. Effect of W-7 on the O_2^--generating responses. Untreated (hatched bars) and Ca^{2+}-depleted macrophages (open bars) were incubated with W-7 for 5 min at 37℃, and then stimulated by PMA, and through $Fc\gamma_1/\gamma_2R$ and $Fc\gamma_2R$, as described in METHODS; the final concentration of W-7 added was 5μM. The results are expressed as percent of control values in the absence of W-7. Each value represents the mean ± SEM. The control values obtained with untreated macrophages were 8.5, 1.2 and 2.4 nmol/min/10^6 cells for the PMA-induced, and $Fc\gamma_1/\gamma_2R$- and $Fc\gamma_2R$-triggered O_2^- generation, respectively, and their counterparts of Ca^{2+}-depleted macrophages were 8.3, 0.6 and 2.4 nmol/min/10^6 cells, respectively.

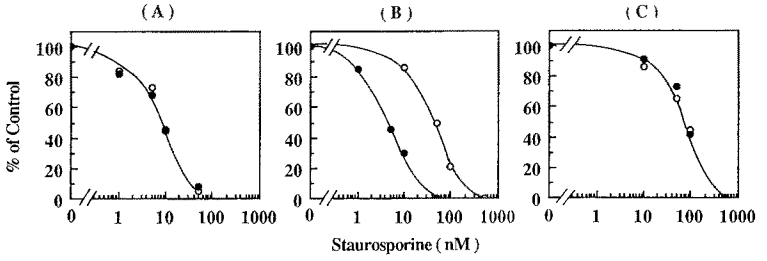

Fig.2. Inhibition of the O_2^--generating responses with staurosporine. Untreated (○) and Ca^{2+}-depleted (●) macrophages were incubated with various concentrations of staurosporine for 5 min at 37℃, and then stimulated by PMA (A), and through $Fc\gamma_1/\gamma_2R$ (B) and $Fc\gamma_2R$ (C), as described in METHODS. The results are expressed as percent of control values in the absence of staurosporine. The control values obtained with untreated macrophages were 7.2, 0.79 and 2.2 nmol/min/10^6 cells for the PMA-induced, and $Fc\gamma_1/\gamma_2R$- and $Fc\gamma_2R$- triggered O_2^- generation, respectively, and their counterparts of Ca^{2+}-depleted macrophages were 7.0, 0.38 and 2.5 nmol/min/10^6 cells, respectively.

therefore, examined the effect of staurosporine on the O_2^--generating responses triggered by the FcγRs. The dose-inhibition curves obtained showed that all the O_2^--generating responses examined were strongly inhibited with staurosporine, but to remarkably different extents (Fig.2). The IC_{50} values indicating concentrations of staurosporine causing 50% inhibition were 7.3, 49 and 80 nM for the responses induced by PMA and those triggered by $Fc\gamma_1/\gamma_2R$ and $Fc\gamma_2R$, respectively (Table 1). Among these IC_{50} values, those for the responses induced by PMA and triggered by $Fc\gamma_2R$ did not vary before and after the Ca^{2+} depletion. However, the IC_{50} value for the $Fc\gamma_1/\gamma_2R$-triggered response was markedly lowered from 49 to 4.7 nM by the Ca^{2+} depletion. This change suggests that two different signal-transduction systems coupled to $Fc\gamma_1/\gamma_2R$, suggested by the experiments with W-7, differ from each other in susceptibility to the inhibitory activity of staurosporine. In the Ca^{2+}-depleted macrophages, the IC_{50} value obtained indicates that protein kinase C (19) may be involved in the signal-transduction system leading to the activation of the NADPH oxidase, as in the case of the PMA-induced system. Actually, we found that the Ca^{2+}-

Table 1. IC_{50} values of staurosporine on the O_2^--generating responses

O_2^--generation	IC_{50} (nM)	
	Untreated cells	Ca^{2+}-depleted cells
PMA-induced	7.3 ± 0.6	6.6 ± 0.7
$Fc\gamma_1/\gamma_2R$-triggered	49 ± 3.3	4.7 ± 0.8
$Fc\gamma_2R$-triggered	80 ± 0.9	83 ± 1.5

Each value was obtained from the results in Fig.2, and represents the mean ± SEM.

depletion did not affect the phosphatidylinositol turnover triggered by Fcγ_1/γ_2R (20). The formation of diacylglycerol, therefore, may occur even in the Ca^{2+}-depleted macrophages, resulting in the activation of protein kinase C. In another system, presumably the Ca^{2+}-calmodulin system, operating in untreated macrophages, a certain protein kinase, which is less sensitive to staurosporine than is protein kinase C, may be involved, as staurosporine is known to inhibit not only protein kinase C but also other protein kinases (21). Some staurosporine-less sensitive protein kinase also seems to operate in the signal-transduction system linked to Fcγ_2R.

The results obtained so far in our laboratory had demonstrated the different abilities of Fcγ_2R and Fcγ_1/γ_2R to induce various functions of guinea pig macrophages; Fcγ_2R triggers the Ca mobilization (4), O_2^- generation (4) and phagocytosis of sensitized sheep erythrocytes (22) much more intensively than does Fcγ_1/γ_2R, although the number of Fcγ_2R molecules per cell is one-half that of Fcγ_1/γ_2R (22). The phagocytosis of OA complexes of IgG antibodies, on the other hand, had been found to proceed more effectively through Fcγ_1/γ_2R than Fcγ_2R (23). Based on these differences, we proposed that the signal-transduction systems coupled to these FcγRs may differ from each other. The results described in this paper support our proposition.

Acknowledgment: This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Shimamura,T., Nakamura,T. and Koyama,J. (1986) J. Biochem. 99, 227-235.
2. Shimamura,T., Nakamura,T. and Koyama,J. (1987) Molec. Immun. 24, 67-74.
3. Tominaga,M., Sakata,A., Ohmura,T., Yamashita,T., Koyama,J., and Onoue K. (1990) Biochem. Biophys. Res. Commun. 168 683-689.
4. Imamichi,T., Sato,H., Iwaki,S., Nakamura,T. and Koyama,J. (1990) Molec. Immun. in press.
5. Shinomiya,T. and Koyama,J. (1976) Immunology 30, 267-275.
6. Nakagawara,M., Takeshige,K., Sumimoto,H., Yoshitake,J. and Minakami,S. (1984) Biochim. Biophys. Acta. 805, 97-103.
7. Grynkiewicz,G., Poenie,M. and Tsien,Y.R. (1985) J. Biol. Chem. 260, 3440-3450.
8. Tamoto,K. and Koyama,J. (1976) Immunology 31, 67-77.
9. Washida,N., Sagawa,A., Tamoto,K., and Koyama,J. (1980) Biochim. Biophys. Acta. 631, 371-379.
10. Kobayashi,R., Tawata,M., and Hidaka,H. (1979) Biochem. Biophys. Res. Commun. 88, 1037-1045.
11. Hidaka,H., Yamaki,T., Naka,M., Tanaka,T., Hayashi,H. and Kobayashi,R. (1980) Mol. Pharmacol. 17, 66-72.
12. Hidaka,H., Naka,M. and Yamaki,T. (1979) Biochem. Biophys. Res. Commun. 90, 694-699.

13. Tanaka,T., Ohmura,T., Yamakado,T. and Hidaka,H. (1982) Mol. Pharmacol. 22, 408-412.
14. Wise,B.C., Glass,D.B., Chou,C-H.I., Raynor,R.L., Katoh,N., Schatzman,R. C., Turner,R.S., Kibler,R,F. and Kou,J.F. (1982) J. Biol. Chem. 257, 8489-8495.
15. Tamaoki,T., Nomoto,H., Takahashi,I., Kato,Y., Morimoto,M. and Tomita F. (1986) Biochem. Biophys. Res. Commun. 135, 397-402.
16. Sako,T., Tauber,A.I., Jeng,A.Y., Yuspa,S.H. and Blumberg,P.M. (1988) Cancer Res. 48, 4646-4650.
17. Dewald,B., Thelen,M., Wymann,M.M. and Baggiolini,M. (1989) Biochem. J. 264, 879-884.
18. Combadière,C., Hakim J., Giroud,J.P. and Périanin,A. (1990) Biochem. Biophys. Res. Commun. 168,65-70.
19. Nishizuka,Y. (1984) Nature 308, 693-698.
20. Imamichi and Koyama (1990) Molec. Immun. in press.
21. Rüegg,U.T. and Burgess,G.M. (1989) Tips 10, 218-220.
22. Nakamura,T., Sato,H., Shimamura,T. and Koyama,J. (1988) J. Biochem. 104, 383- 387.
23. Sato,H., Nakamura,T. and Koyama J. (1989) Molec. Immun. 25, 205-211.