Isolation of Fc receptor shed from pig lymphocytes by a temperature shift

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Received 4 April 1984

Lymphocytes obtained from pig blood by gradient centrifugation were subjected to a temperature shift (4 to 37°C). The proteins released from the plasma membrane were fractionated by affinity chromatography using immunoglobulin G immobilized on fine polyamide particles. The main component liberated from the adsorbent by diethylamine buffer (pH 11.5) exhibited an apparent M_r of 18000–20000 in SDS–polyacrylamide gel electrophoresis. This crude receptor preparation possessed a substantially higher affinity to immobilized immunoglobulin G than to immobilized Fab fragment and inhibited significantly the binding of labeled immunoglobulin G to pig lymphocytes.

Fc receptor *Pig immunoglobulin* *Proteolytic fragment* *Pig lymphocyte* *Affinity chromatography*

1. INTRODUCTION

Receptors for the Fc portion of immunoglobulin molecules occur in plasma membranes of numerous cell types in all vertebrate organisms (review [1,2]). Most Fc receptors are specific for the immunoglobulin class and some also for IgG subclasses. Numerous functional studies have been published that elucidate the binding properties and specificity of various Fc receptors. Since biochemical studies of isolated Fc receptors are still scarce, an effort was made to purify Fc receptor for IgG from lymphocytes of an animal species the blood of which is easily available in large quantities. Pig lymphocytes were found suitable for this study, not least because the preparation of proteolytic fragments of pig IgG has been elaborated [3–5].

Abbreviations: PBS, phosphate-buffered saline (150 mM NaCl, 20 mM NaH_2PO_4 buffered to pH 7.4); IgG, immunoglobulin G; Fab, Fc, pFc', proteolytic fragments of IgG; BSA, bovine serum albumin; PSA, pig serum albumin; PMSF, phenylmethylsulfonyl fluoride; NP40, Nonidet P40

2. MATERIALS AND METHODS

2.1. *Materials*

Nonspecific pig IgG, its *S*-sulfo derivative, tryptic fragments Fab and Fc and peptic fragment pFc' were prepared as in [3–5]. Radioactively labeled IgG was prepared by reductive methylation [6] using [^{14}C]formaldehyde (Amersham, England). Fine porous particles of alkalic polyamide (prepared by V. Kubánek, Institute of Chemical Technology, Prague) were used as a carrier of IgG, Fab and BSA. The proteins were immobilized on the carrier particles by glutaraldehyde (to be published). Solutions of dextran M_r 30000–40000 (Dextran, Spofa, Czechoslovakia) and of sodium 3,5-diacetamido-2,4,6-triiodobenzoate (Verografin, Spofa, Czechoslovakia) were preparations for medical use.

2.2. *Peripheral blood lymphocytes*

Pig blood (20 l) was mixed with 8.4 l anticoagulant solution (2.43% sodium citrate, 0.26% NaCl, 1.71% dextran). Erythrocytes were removed by centrifugation at 400 × *g*; leukocytes were

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00145793/84/$3.00

sedimented at 650 × *g* and washed by washing solution composed of PBS (5 vols) and anticoagulant solution with 0.5% glucose (2 vols). Lymphocytes were separated by density gradient centrifugation (700 × *g*) employing a bottom layer formed by 22.5% Verografin. The cell suspension obtained consisted of more than 97% lymphocytes. The viability checked by trypan blue exclusion was 95–98%.

2.3. *Temperature shift-induced shedding*

The procedure was carried out essentially as in [7]. After 30 min incubation at 4°C the suspension was gently shaken for 1 h at 37°C. The supernatant was supplemented by 0.02% NP40, PMSF (2 μg/ml) and concentrated by ultrafiltraiton using an Amicon YM-5 membrane. The solution was stored at −20°C.

2.4. *Affinity chromatography*

To reduce the content of nonspecifically binding components the solution of shed proteins (40–50 ml) was first shaken with 1.5 g immobilized BSA (4°C, 30 min). The supernatant was then shaken with 1.5 g immobilized IgG overnight at 4°C. The Fc receptor was desorbed from the washed adsorbent by 50 mM diethylamine–HCl buffer (pH 11.5) with 0.1% NP40. The Fc receptor solution was neutralized without delay.

2.5. *Binding assays*

Lymphocytes were preincubated for 2 h at 20°C in RPMI 1640 medium containing 0.05% PSA to remove IgG bound to receptors [8]. A volume (50 μl) of RPMI 1640 medium with 0.02% NaN_3 and 0.4% PSA, containing 2×10^7 lymphocytes, was supplemented by 48 μl of the solution of competitor and 2 μl of the solution of [^{14}C]IgG. The suspension was shaken at 20°C for 30 min. The cells were centrifuged, washed twice with PBS and the radioactivity measured by scintillation counting in Bray solution.

2.6. *Electrophoresis*

Gradient (4–30%) SDS–polyacrylamide gel electrophoresis employing essentially the discontinuous buffer system of [9] was used. The samples were analyzed without mercaptoethanol. Gels were fixed with 50% trichloroacetic acid and the proteins stained with silver [10].

3. RESULTS AND DISCUSSION

3.1. *Isolation and characterization of shed Fc receptor*

Lymphocytes from four 20-l portions of blood served as a source of receptor for its preliminary characterization and assay of its specificity. The total quantity of protein obtained after shedding was 100–160 mg per 10^{10} cells. Upon affinity chromatography the quantity of material, that may be classified as the crude receptor, was about 1% of total shed protein. The quantity of protein irreversibly bound to the adsorbent was in the range 1–3%.

The crude receptor was tested for its specific binding capacity in analytical affinity chromatography experiments using both immobilized IgG and immobilized Fab fragment. In several experiments carried out with immobilized IgG 60–70% of the sample was adsorbed and could be recovered with diethylamine buffer, whereas the corresponding figure for experiments with immobilized Fab was 15–20%. The relatively low figure with IgG indicates that the crude receptor contains impurities and/or denatured material.

The electrophoretic analysis (fig.1) showed that the crude receptor consists of one main component

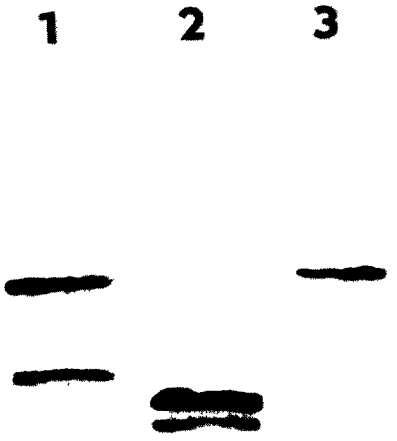

Fig.1. Gradient SDS-polyacrylamide gel electrophoresis of the Fc receptor. Lane 1, *S*-sulfo IgG (heavy chain M_r 50000, light chain M_r 22500); lane 2, preparation of the Fc receptor obtained by affinity chromatography; lane 3, BSA (M_r 66000).

Table 1

Inhibition of binding of [^{14}C]IgG to pig lymphocytes by various specific competitors

Competitor	Concentration (M) ($\times 10^5$)	Bound [^{14}C]IgG (cpm)	Inhibition (%)
None	–	1410	0
IgG	1.23	660	53
IgG	4.99	610	51
Fc	4.92	770	45
pFc′	1.23	910	35
pFc′	4.92	930	34
Fc receptor	0.11	690	51
Fc receptor	0.46	540	62
Fab	4.92	1450	0

Each assay was carried out with 2×10^7 cells. Concentration of [^{14}C]IgG was 2.46×10^{-7} M

of about 18–20 kDa and a minor band with a lower molecular mass. Trace impurities (not visible in fig.1) with higher molecular masses were present as well. The apparent molecular mass of the main component of the crude Fc receptor is lower than most of the previously reported values for various Fc receptors [1,2]. The specific feature of the receptor investigated here is its loose connection to the plasma membrane resulting in its easy shedding. Fc receptor with similar properties has also been described in [11]. The shed receptors may be related to or even be identical with immunoglobulin-binding factors produced by T-cell hybrids [12]. At the present stage it cannot be ruled out that the 18-kDa component represents a breakdown product of a larger native receptor.

3.2. *Specificity of binding*

The binding of labeled IgG to pig lymphocytes was found to be inhibited by unlabeled IgG, Fc and pFc′ fragments and by the isolated soluble Fc receptor. No inhibition at all was observed with the Fab fragment (table 1). This result is consistent with the view that the isolated receptor is identical with at least some of the receptors that operate in the plasma membrane of pig lymphocytes. The membrane receptor obviously also binds Fc and pFc′ fragments of IgG, because both these substances display a certain inhibition effect.

Even though some unanswered questions remain concerning the purity and native form of the shed Fc receptor, the result of the binding assays strongly suggests that the isolated receptor carries the receptor binding site.

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