IDENTIFICATION OF A RECEPTOR FOR IgG ON HUMAN GRANULOCYTES

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1. Introduction

Human neutrophils carry on their membrane a receptor for the Fc portion of IgG [1]. Electron microscopic studies show that the receptors appear as clusters on the neutrophil membrane [2]. On infection, the neutrophil recognises and binds the antibody carrying the foreign material and disposes off the complex by a combination of phagocytosis, bacteriocidal activity and digestion.

The receptors for IgG also vary in number depending on the state of maturation of the cell [3,4]. Hence it is a maturation marker of the granulocytic series.

One approach to completely understand the role of the receptor for IgG at the cell surface is to isolate and biochemically characterize the molecule. The receptor for IgG on human B lymphocytes has been isolated and characterized [5–7]. However, there are no reports on the identification and chemical structure of the granulocyte molecule. Here, we have identified and partially characterized the IgG receptor from the granulocyte membrane.

2. Materials and methods

2.1. *Materials*

All chemicals used were of the best grade available.

2.2. *Preparation of granulocytes*

Normal 'O' group blood was collected in acid citrate dextrose solution from voluntary donors at the Tata Memorial Hospital, Bombay. Granulocytes and erythrocytes were separated from other cells by the Ficoll-Hypaque gradient centrifugation according to [8]. The pellet containing granulocytes and erythrocytes was suspended in 0.83% NH_4Cl/0.172 M Tris–HCl (pH 7.6) (9:1, v/v) and kept at 37°C for 5 min to lyse the red blood cells. The granulocytes were separated by centrifugation and finally washed twice with 0.172 M Tris–HCl (pH 7.6).

2.3. *Preparation of human IgG*

The γ-globulin fraction was precipitated from human plasma with 33% ammonium sulphate, and purified further by chromatography on DEAE-cellulose. The purity of the preparation was checked by SDS–polyacrylamide gel electrophoresis (SDS–PAGE).

2.4. *Preparation of crude membrane pellet*

Granulocytes (1 ml pack) were suspended in 20 ml buffer A [0.01 M Tris–HCl (pH 7.6)/0.1 mM phenylmethylsulfonyl fluoride (PMSF)], for 6 min and 20 ml 0.25 M sucrose in buffer A was then added. The cells were disrupted by passing 5 times through a 10 ml syringe carrying a 4 cm long no. 20 gauge needle. [Sucrose] in this homogenate was adjusted to 0.32 M by adding 10 ml 38% sucrose in buffer A. Nuclei and unbroken cells were removed by centrifugation at 1100 × *g*. The supernatant was spun at 13 000 rev./min (20 000 × *g*) for 40 min to obtain the crude membrane pellet P_1.

2.5. *Solubilization of membranes by Triton X-100*

The crude membrane pellet P_1 was suspended in 0.038 M Tris/0.1 M glycine/0.1 mM PMSF/0.5% Triton X-100 (Triton buffer) at 1–2 mg/ml buffer and stirred overnight at 4°C. The mixture was then centrifuged at 40 000 rev./min (100 000 × *g*) for 1 h. The supernatant was used for further analysis and the pellet discarded.

2.6. *Precipitation of the receptor for human IgG from the solubilized membranes*

IgG (200 μg) was added to 1–3 mg solubilized

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membrane in 2.5 ml of Triton buffer and the mixture kept at room temperature for 1 h. Goat antiserum to human γ-chain (3 mg in 50 μl) was then added and the mixture kept for another 1 h at room temperature. The precipitate formed was removed by centrifugation at 10 000 rev./min (10 000 × *g*) for 10 min. A control containing 2.5 ml Triton buffer instead of the membrane extract was also processed identically. The precipitates were washed thrice with the Triton buffer. They were finally solubilized in sample buffer containing SDS with or without mercaptoethanol and subjected to SDS–PAGE on 10% or 7.5% acrylamide gels.

2.7. *Electrophoresis*

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis was done as in [9]. The slab gels (1.5 mm thick) were run at 23 mA till the marker dye reached the end of the gel. The gels were stained in 0.2% Coomassie blue in 50% methanol–10% acetic acid and finally destained with 50% methanol–10% acetic acid. They were stored in 10% acetic acid. Gels were stained for carbohydrate containing protein by the periodic acid Schiff stain according to [10].

2.8. *Protein estimation*

Protein was estimated according to [11]. Blanks containing equivalent volume of Triton buffer were made where necessary.

3. Results

3.1. *Solubilization*

Overnight stirring of the crude membrane pellet P_1 (~1–2 mg protein/ml) in Triton buffer resulted in partial solubilization of the membrane components. The supernatant obtained after centrifugation at 100 000 × *g* contained an av. 700 μg protein/ml.

3.2. *Coprecipitation*

Human IgG (200 μg) was added to the Triton extract containing 1–3 mg protein. After 1 h at room temperature the complex was precipitated with goat antiserum to human γ-chain. The precipitate was washed thrice with Triton buffer and then subjected to SDS–PAGE. A control precipitation using only Triton buffer instead of the extract was treated similarly. Fig.1 shows the results of the electrophoresis. Lane A is the sample and lane D the control. In lane A there are 2 prominently stained proteins corresponding to the heavy chain and light chain of IgG and a third band between them. This band is absent in the control and corresponds to the IgG binding protein. In the control there is a band above the heavy chain of IgG. This band is faint in the sample and can be eliminated by increased number of washes of the precipitate. It is most likely a protein component from goat antiserum which has an affinity for human IgG. In the sample, the human IgG is already bound by its receptor from the extract. It can, therefore, only bind very weakly to the goat protein and is removed by repeated washing. Fig.2 shows a densitometer scan of the gel in fig.1. The M_r of the IgG binding protein is 33 880 as calculated from its position on the gel and the mobility of the M_r standards, bovine serum albumin and ovalbumin.

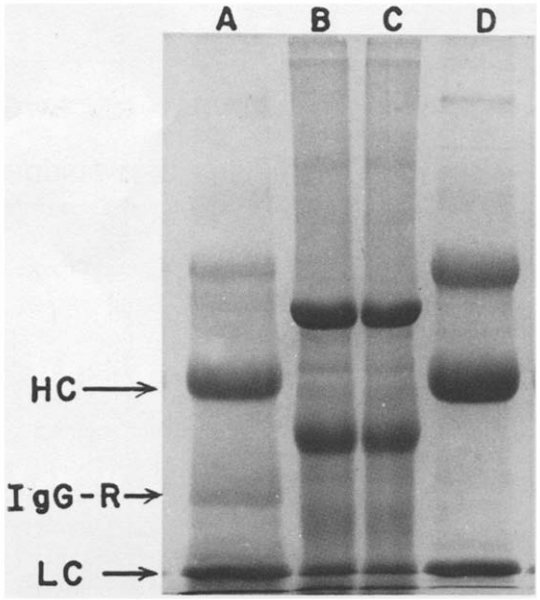

Fig.1. SDS–polyacrylamide gel (10%) pattern of the IgG-receptor–anti-IgG coprecipitates: (A) sample; (D) control. The standards bovine serum albumin and ovalbumin are in both the (B,C). The position of the IgG-receptor (IgG-R), heavy chain (HC) and light chain (LC) of IgG are shown.

Experiments using increasing amounts of the Triton X-100 extract with constant amounts of IgG and anti IgG show that there is a quantitative increase in the 33 880 M_r band. This is shown in fig.3 which depicts the densitometric scan of an experiment in which 0.7 mg and 1.4 mg Triton extract were used. The ratio of the area under the 33 880 M_r peak and

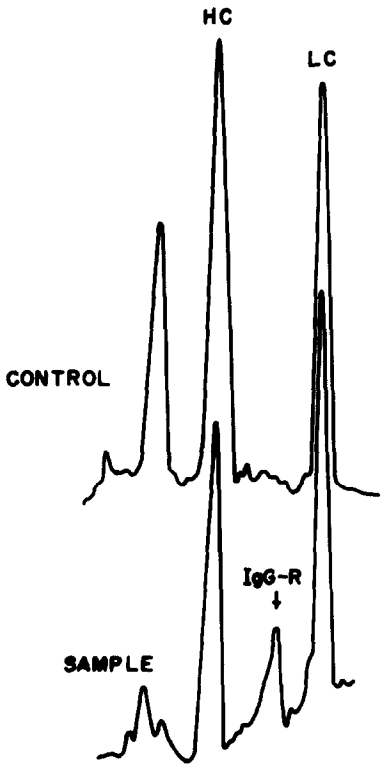

Fig.2. Densitometer scans of the 10% SDS–polyacrylamide gels shown in fig.1. The position of the IgG-receptor (IgG-R) is indicated by the arrow. HC and LC stand for the heavy and light chain of IgG, respectively.

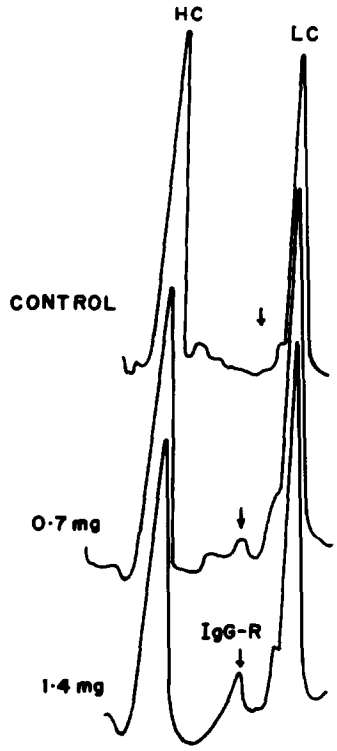

Fig.3. Densitometer scans of the 10% SDS–polyacrylamide gels showing the increase of the IgG-R band with increasing amounts (0.7 mg and 1.4 mg) of the membrane extract used for the coprecipitation. The position of the IgG-receptor is indicated by the arrow. There is no peak in this position of the control.

the area of the heavy chain is 0.04 for the 0.7 mg run while is is 0.078 for the 1.4 mg run. This suggests that the M_r 33 880 moiety is definitely being obtained from the membrane extract.

Repeated experiments have shown that the M_r 33 880 band is broken down into a M_r 29 000 band and PMSF does not prevent this break down completely. Fig.4 shows the densitometer scan of an experiment in which Triton X-100 extracts containing 1.5 mg protein were used for precipitation. Sample A has only a single band at M_r 33 880, while sample B shows break down products. The band at M_r 33 880 becomes diffuse and another band appears at M_r 29 000.

SDS–PAGE profiles of the coprecipitate dissolved in buffer not containing mercaptoethanol is shown in fig.5. A single band corresponding to M_r 74 990 is seen in lane C which is absent from the 2 control samples in lanes A and B. This suggests that the receptor for IgG probably exists as a dimer which is held by disulfide bonds.

Periodic acid Schiff staining of a gel in which the sample was reduced with mercaptoethanol shows a very faint band of M_r 29 000.

4. Discussion

Our experiments show that human IgG binds a protein component in the Triton X-100 extract of the crude membrane pellet of human granulocytes. This complex can be coprecipitated with goat antiserum to human γ-chain. The precipitate when solubilized in buffer containing SDS and mercaptoethanol and analysed in SDS gels shows a distinct protein band at M_r 33 880 as computed from the internal standards bovine serum albumin (M_r 68 000), ovalbumin (M_r 43 000), heavy chain of IgG (M_r 50 000) and light chain of IgG (M_r 25 000). This band is very susceptible to proteolysis and as a result breaks down into a protein component of ~29 000 M_r. The coprecipitate when dissolved in SDS buffer not containing mercaptoethanol and analysed on SDS–PAGE, shows

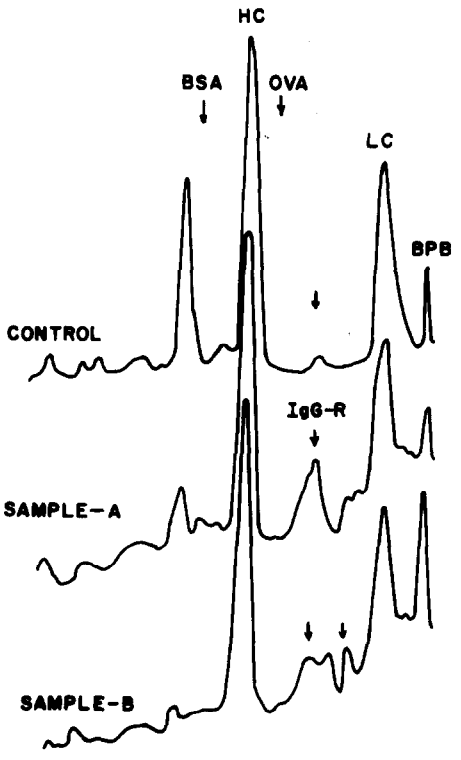

Fig.4. Densitometer scans of the 10% SDS–polyacrylamide gels showing the IgG-receptor and its breakdown products. Positions of bovine serum albumin (BSA), ovalbumin (OVA), heavy chain (HC) and light chain (LC) of IgG and bromophenol blue (BPB) are shown. In (A) there is one major band indicated by IgG-R. In (B) this band becomes diffuse and another band appears at M_r 29 000.

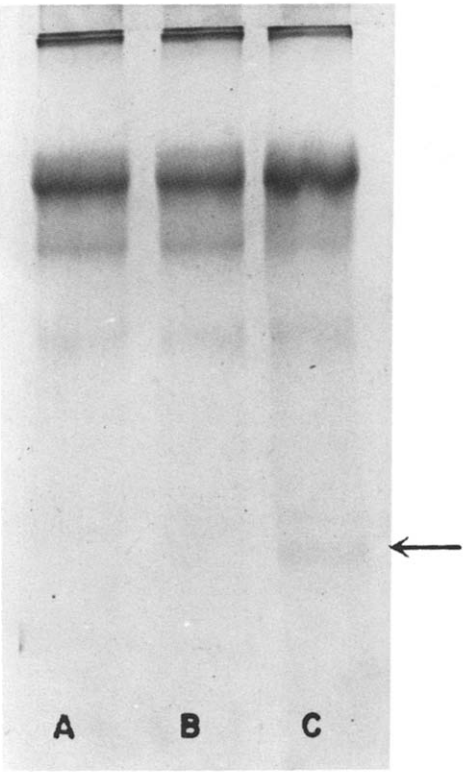

Fig.5. SDS–polyacrylamide gels (7.5%) patterns of unreduced IgG-receptor–anti-IgG coprecipitates: (A,B) control; (C) sample. The position of the unreduced IgG-R (M_r 74 990) in (C) is indicated by an arrow. There is no corresponding band in the controls. The most prominent band in (A–C) is unreduced IgG (M_r 150 000).

a band at M_r 74 990. This suggests that the granulocyte membrane receptor for IgG probably exists as a dimer.

The receptor for IgG on B lymphocytes has been identified [5–7]. The results in [6] suggest that it is a phospholipoprotein of ~30 000 M_r and most likely consists of a single chain. In [7] the B cell receptor of 28 000 M_r formed a tetramer in the absence of mercaptoethanol. The granulocyte receptor appears to be similar to the receptor isolated from human lymphocytes.

Information on the biochemical nature of this membrane component is a step towards understanding the nature of the association of this interesting functional protein with the cell membrane. The vicinal relationship of this protein with others to form functional units remain challenging problems in cell biology.

References

[1] Messner, R. P. and Jelinek, J. (1970) J. Clin. Invest. 49, 2165–2171.
[2] An, T. (1980) Immunology 40, 1–8.
[3] Burns, G. F. and Cawley, J. C. (1979) Brit. J. Haematol. 42, 499–505.
[4] Harlozinska, A., Potomski, J., Noworolska, A., Becker, M. and Richter, R. (1980) Scand. J. Haematol. 25, 205–213.
[5] Suzuki, T., Sadasivan, R., Wood, G. and Bayer, W. L. (1980) Mol. Immunol. 17, 491–503.
[6] Suzuki, T., Taki, T., Hachimine, K. and Sadasivan, R. (1981) Mol. Immunol. 18, 55–65.
[7] Thones, J. and Stein, H. (1979) Adv. Exp. Med. Biol. 114, 199–205.
[8] Böyum, A. (1974) Tiss. Antig. 4, 269–274.
[9] Laemmli, U. K. (1970) Nature 227, 680–685.
[10] Neville, D. M. and Glossman, H. (1974) Methods Enzymol. 32B, 92–102.
[11] Hartree, E. F. (1972) Anal. Biochem. 48, 422–427.