ABSTRACT

One of the important lymphocyte membrane protein is immunoglobulin receptor which specifically recognizes the Fc portion of the IgG molecule. The membrane receptor has been identified on variety of cells including lymphocytes, monocytes, polymorphonuclear cells and are implicated in functions such as antibody dependent cell mediated cytotoxicity, suppression of antibody synthesis in B cells and phagocytosis in macrophages.

Although IgG receptors obtained from different sources have been found to show species dependent differences in pH stability and structure, studies on receptors from species other than human, murine and guinea pig are yet to be carried out. Further, despite the known functional significance of self association of the receptor, its aggregating tendency in aqueous buffer devoid of detergent remains to be systematically investigated. With this aim in view, we isolated and purified receptor from hitherto uninvestigated source i.e. goat peripheral blood lymphocytes. After its purification and characterization its tendency to undergo aggregation in aqueous medium was studied at different pH and ionic strength.

The presence of IgG binding protein on goat peripheral blood lymphocyte was detected by the specific
binding of FITC conjugated aggregated IgG to lymphocytes in 10 mM sodium phosphate buffer pH 7.4 containing 0.15 M NaCl, 1% BSA and 0.2% NaN₃. The binding of FITC conjugated aggregated IgG to goat peripheral blood lymphocytes increased with the increase in the concentration of aggregated IgG from 2 to 9 μg. Saturation in binding sites on 4.5 x 10⁷ cells could be achieved with 0.7 μg/ml of aggregated IgG.

Binding of human IgG monomer, aggregated IgG and F(ab₂') fragment to peripheral goat blood lymphocytes was studied by ELISA where the IgG or its derivatives bound to the lymphocyte surface receptors were assayed by using peroxidase conjugated F(ab₂') of antihuman IgG under different conditions of pH and ionic strength. The binding of IgG and its derivatives was studied at different pH values in the pH range 3-8 which was maintained either by 0.06 M sodium acetate (pH 3-5) or by 0.06 M sodium phosphate (pH 6-8). It was found that maximum binding occurred with heat aggregated IgG followed by IgG monomer and its fragments F(ab₂').

Furthermore the pH dependence of aggregated IgG was found to be more pronounced than the binding of IgG monomer to cells in the entire pH range (pH 3-8). Maximum binding of aggregated IgG took place at pH 6.0 below or above which the change in pH caused considerable
reduction in binding. The binding of monomeric IgG decreased gradually and monotonically as the pH decreased from pH 8 to pH 3. A small but experimentally significant binding of F(ab'_2) to cells was observed; it was completely abolished below pH 4.0.

The effect of ionic strength on binding of IgG and its derivatives to goat peripheral blood lymphocytes was studied in 10 mM phosphate buffer pH 7.6 at 37°C. Increasing concentration of NaCl up to 0.8 M caused significant decrease in the binding. The ionic strength dependence of the binding was more pronounced with heat aggregated IgG.

Goat peripheral blood lymphocyte receptor for IgG was isolated by affinity chromatography both from cell homogenate as well as from isolated membrane. The lymphocyte membrane was prepared by hypotonic lysis of cells by freezing in 10 mM tris HCl buffer pH 7.0 containing 1 mM MgCl₂ and 1 mM KCl followed by centrifugation. The membrane fraction was identified by the measurement of 5'-nucleotidase activity against AMP in 55 mM Tris HCl buffer pH 8.5 containing 5.5 mM magnesium chloride. The membrane fraction showing a 5'-nucleotidase activity of 1.27 μg Pi/mg/min which was expectedly higher than 0.33 μg Pi/mg/min measured for cell homogenate was used in the isolation of the receptor.
The membrane or the whole cell were solubilized in 10 mM sodium phosphate buffer pH 7.4 containing 0.15 M NaCl, 0.5% NP-40, 2 mM PMSF, 3 mM EDTA and 10 mM iodoacetamide and after treating the mixture with BSA Sepharose the protein was incubated with heat aggregated IgG Sepharose 4B affinity column. The protein yield of the affinity purified receptor was less than 1%. The receptor preparations, both from the isolated membrane and whole cells were identical in (a) reactivity towards IgG aggregated and Con A Sepharose gel (b) gel filtration behaviour (c) subunit molecular weight and (d) finally in their tendencies to undergo pH and temperature dependent aggregation.

The receptor preparation was homogeneous with respect to size as indicated by SDS-PAGE where it moved as a single protein band with a subunit molecular weight of 14 kDa both in the presence and absence of 0.2 M mercaptoethanol. These results suggest that the subunits in the receptor are held together only by noncovalent forces.

The IgG binding receptor was found to be a glycoprotein devoid of sialic acid residues. The hexose content of the receptor as determined by phenol sulphuric acid method was found to be 11% (w/w) i.e. about 9 moles of hexose per 14000 g of the receptor. As the receptor
specifically interacted with Con A-Sepharose column its carbohydrate moieties must include specific sugar residues such as mannose/glucose. In 10 mM sodium phosphate buffer plus 0.15 M NaCl and 0.1% deoxycholate the IgG receptor absorbed maximally at 278 nm. The measured fluorescence excitation and emission spectra of the receptor in the same buffer showed excitation and emission maxima near 277.6 nm and 341.4 nm, respectively. These results suggest that the aromatic amino acid residues of the receptor must include tryptophan residue(s).

On gel filtration of the IgG binding receptor at 25°C on an HPLC Shim Pack Diol 150 column equilibrated in 10 mM sodium phosphate buffer pH 7.4 containing 0.5 M NaCl, 60% of the receptor eluted with a retention time of 6.46 min which yielded a molecular weight of 67000 and Stokes radius of 3.48 nm. Other hydrodynamic parameters that were calculated from the Stokes radius included diffusion coefficient (5.2x10^{-7} cm^2/sec) and frictional ratio (1.3). These data are consistent with a nonglobular conformation of the major fraction of the receptor in aqueous buffer solution devoid of detergent. The minor fractions included higher aggregates with molecular weights of 119 kDa and 94 kDa.

The IgG binding receptor showed pH and temperature dependent association. In 0.5 M acetic acid pH 2.8, and at
37°C the receptor was found to exist primarily as 31 kDa species. On lowering temperature, at the same pH, to 4°C association of 31 kDa species into 67 kDa species occurred. The trimer and tetrameric forms of 31 kDa species which were absent in 0.5 m acetic acid pH 2.8 at 37°C appeared in 10 mM sodium phosphate, 0.5 NaCl pH 7.4 at 25°C.

Thus both acidic pH as well as increase in temperature favoured dissociation of IgG binding protein to 31 kDa from. As revealed by SDS-PAGE results the 31 kDa species itself seems to be a dimer of 14 kDa subunits held together by noncovalent interactions. Since increase in temperature favours dissociation of the goat IgG binding receptor the self association of the goat lymphocyte receptor is likely to be exothermic in nature.