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Immunology

IgM receptors on human lymphoctyes: Detection by direct binding

(8S IgM/cytophilic immunoglobulins/helper cells)

JOHN A. HARDIN, KURT K. NAKAOKA*, AND JOAN M. CARBONI

Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06510

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ABSTRACT **Radioiodinated IgM isolated from a patient with Waldenström macroglobulinemia binds with high avidity (apparent $K_a = 2.5 \times 10^9\ M^{-1}$) to freshly isolated human peripheral blood lymphocytes. Binding occurred through the Fc region of the molecule and involved a receptor specific for immunoglobulins of the IgM class. The avidity and specificity of this binding are consistent with a biologic role for IgM receptors on lymphocytes.**

Human T lymphocytes with helper cell activity (1–5), and some B lymphocytes (6), bear receptors specific for the Fc portion of IgM proteins. Identification of such lymphocytes has been based upon their ability to form rosettes with ox erythrocytes coated with antibodies of the IgM class. This method is cumbersome and requires that cells be incubated in serum-free media overnight, presumably to free their receptors of endogenously bound ligands. From a thermodynamic point of view, IgM molecules free in solution should compete for cell surface receptor sites much more effectively than IgM bound to erythrocytes. In this study, we show that a human IgM protein will bind directly to freshly isolated human lymphocytes by a region in its Fc domain, and that this binding is inhibited specifically by IgM from normal serum.

METHODS

IgM proteins were isolated by Pevikon block electrophoresis of sera obtained from normal donors, or from the thrice-formed euglobulin fraction of sera from patients with Waldenström macroglobulinemia, and then purified further by two passages over Sephadex G-200 followed by elution from DEAE-Sephadex A-50. Purified proteins were free of IgG, IgA, and albumin by double immunodiffusion; on sodium dodecyl sulfate/10% polyacrylamide slab gel electrophoresis (7), only μ heavy chains and immunoglobulin light chains were observed. The proteins were iodinated to a specific activity of approximately 1.3 μCi/pmol (48,000 becquerels/pmol) by the lactoperoxidase method (8). After density gradient ultracentrifugation, radioactivity was detectable only in a 19S peak.

In preliminary tests of four monoclonal IgM proteins, the greatest binding to lymphocytes occurred with IgM(AR) (light chain type κ). This protein was selected for study. Fc(5μ) fragments of IgM(AR) were prepared by the method of Plaut and Tomasi (9). Twenty milligrams of IgM(AR) was digested with 0.4 mg of trypsin (Miles) for 3 hr at 60°C in 15 mM $CaCl_2$/50 mM Tris· HCl, pH 8.1. The reaction was stopped by the addition of 0.4 mg of soybean trypsin inhibitor (Mann Research Laboratories, New York, NY), and the Fc(5μ) fragments were recovered by gel filtration. The purified Fc(5μ) fragments reacted in gel diffusion with anti-μ-chain antisera but not with anti-light-chain antisera. F(ab')$_2$ μ fragments of IgM(AR) were prepared by the method of Mihaesco and Seligmann (10). Ten milligrams of IgM(AR) was digested with 0.2 mg of pepsin (Sigma) for 4 hr at 37°C in 0.1 M acetate buffer, pH 4.5. The reaction was stopped by neutralization with a saturated Tris solution, and the F(ab')$_2$ μ fragments were isolated by gel filtration. The purified F(ab')$_2$ μ fragments were composed of intact κ light chains and a μ heavy-chain fragment by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. No intact μ chains were present. IgG was prepared from normal human serum by affinity chromatography, and Fc fragments of IgG were prepared by papain cleavage (11).

Human peripheral blood mononuclear cells were obtained from normal donors by the Ficoll-Hypaque technique as described (12). Phagocytic cells were removed by adherence to plastic petri dishes during incubation in tissue culture medium 199 containing 5% fetal calf serum. The lymphocytes were than washed three times in Hanks' balanced salt solution.

In the design of these experiments, we avoided prolonged wash procedures which might have permitted bound immunoglobulin to dissociate from cell surface receptors, and harsh iodination methods which might have denatured labile proteins. For the binding studies, 0.1–0.3 μg of ^{125}I-labeled IgM(AR) [^{125}I-IgM(AR)] and 12×10^6 lymphocytes were incubated with variable amounts of unlabeled purified immunoglobulins in 0.75 ml of Hanks' solution containing 0.25% bovine serum albumin and 0.1% azide at 4°C. After various periods of incubation, 0.2 ml-aliquots were removed in triplicate, layered over 1 ml of fetal calf serum, and pelleted rapidly in a Beckman model-B Microfuge. The cells were then washed once with cold phosphate-buffered saline, the cell-associated radioactivity was measured, and background radioactivity was subtracted.

RESULTS AND DISCUSSION

Fig. 1*A* shows the time course of the binding of IgM(AR) to lymphocytes. Uptake occurred in a time-dependent manner following first-order reaction kinetics and reached equilibrium after approximately 180 min. At equilibrium, approximately 6×10^{-4} pmol of IgM(AR) was bound per 10^6 lymphocytes. This radioactivity was associated with the cell surface, because nearly all of it could be recovered in the fluid phase after treatment of the cells with trypsin (0.25% in phosphate-buffered saline for 20 min). The binding was cell specific; no binding over background was observed when ^{125}I-IgM(AR) was incubated with polymorphonuclear leukocytes, cells that readily bound radiolabeled IgG in control experiments. The nature of this binding was examined by measurement of the uptake of ^{125}I-IgM in the presence of varying concentrations of unlabeled IgM(AR) (Fig. 1*B*). The Scatchard plot of these data (Fig. 1*C*) was biphasic, indicating at least two types of ligand–cell interactions. The one of higher avidity [apparent association constant (K_a) = $2.5 \times 10^9\ M^{-1}$] was predominant at ligand concentrations below 1×10^{-9} M. At the (extrapolated) point

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* Present address: University of Michigan Medical School, Ann Arbor, MI 48109.

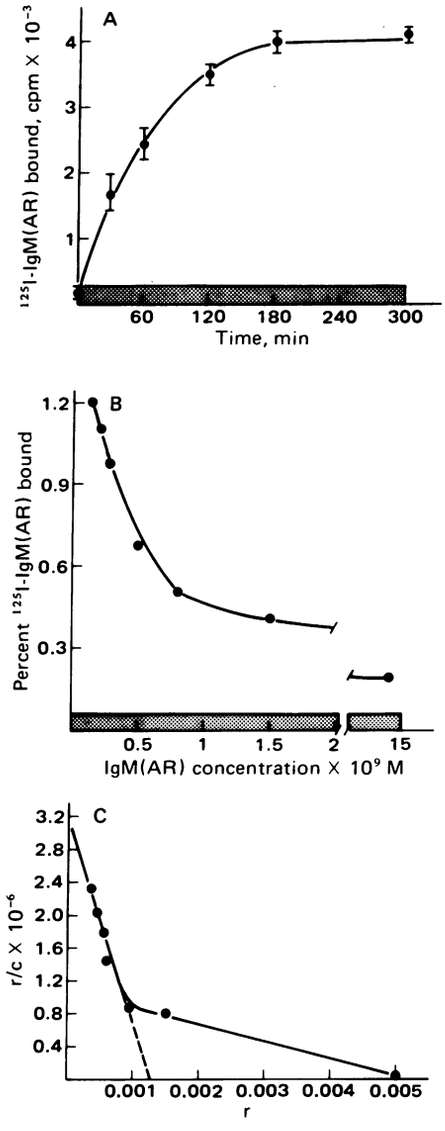

FIG. 1. (*A*) Time course of the binding of ^{125}I-IgM(AR) to lymphocytes. Cultures containing 12 X 10^6 lymphocytes and 0.3 μg of ^{125}I-IgM(AR) in 0.75 ml of medium were incubated at 4°C for the indicated times. Then triplicate 0.2 ml-aliquots were removed and cell bound radioactivity was measured as described. The radioactivity in preparations without cells (shaded area) was subtracted. The range of triplicate determinations is shown. (*B*) Binding of ^{125}I-IgM(AR) to lymphocytes as a function of the molar concentration of IgM(AR). A constant amount of ^{125}I-IgM(AR) (0.1 μg) and various amounts of unlabeled IgM(AR) were incubated with 12 X 10^6 lymphocytes at 4°C for 180 min. Percent of total input of ^{125}I-IgM(AR) bound per 10^6 lymphocytes is shown.

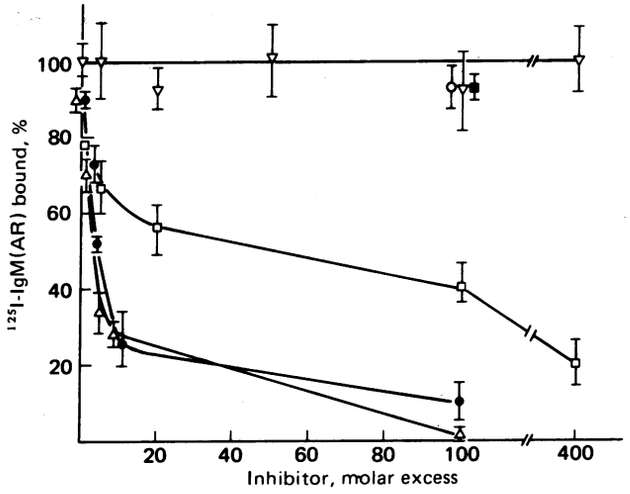

FIG. 2. Inhibition of the binding of ^{125}I-IgM(AR) by various proteins. ^{125}I-IgM(AR) at 0.13 μg/ml (0.14 nM) and various concentrations of unlabeled proteins [shown as molar excess over ^{125}I-IgM(AR)] were incubated with lymphocytes at 4°C for 180 min. The binding is expressed as the percent of ^{125}I-IgM(AR) bound by lymphocytes in the absence of competitive binding protein. △, IgM(AR); ●, IgM (normal); □, Fc(5μ) IgM(AR); ○, IgG; ■, F(ab')$_2$ μ fragments of IgM(AR); ▽, Fc fragments of IgG.

of complete saturation, the high avidity sites were capable of binding approximately 13.3 X 10^{-4} pmol of IgM(AR) per 10^6 cells. Assuming that IgM(AR) has a molecular weight of 900,000 and that each molecule binds to a single receptor, this indicates an average of 804 receptor sites per lymphocyte.

In order to examine the specificity of the binding of ^{125}I-IgM(AR) to lymphocytes, we compared the competitive inhibition produced by adding to the binding mixtures various concentrations of unlabeled IgM(AR), of IgM or IgG from normal serum, or of Fc or F(ab')$_2$ fragments of these molecules. The results, shown in Fig. 2, demonstrate that both IgM(AR) and IgM from normal serum were highly effective as competitive inhibitors of binding. The Fc fragments of IgM(AR) were also effective but to a lesser degree than was the complete molecule, possibly indicating that tertiary structural features important for binding are altered when these fragments are made. No inhibition was produced by the addition of IgG, Fc fragments of IgG, or F(ab')$_2$ μ fragments of IgM(AR).

These data demonstrate that IgM molecules apparently not complexed with antigen can bind by their Fc domains to a specific receptor on at least some human lymphocytes. It seems likely that this receptor is the same as the one detected by rosette formation with IgM-coated ox erythrocytes—both are specific for IgM and both can be blocked by IgM free in solution (1). In the rosette assay, the receptor for IgM is detectable on only about one-half of human peripheral blood T cells, and some B cells. If the receptors that we have measured directly are indeed comparable, then their density on operative lymphocytes may be higher than the average value indicated above.

The relatively small number of receptors may explain why other investigators have failed to detect cytophilic properties among IgM proteins by direct binding methods (13). Also, the method of iodination appears to be important. We were unable to detect binding of IgM(AR) to lymphocytes when the protein was iodinated with chloramine T. Finally, incubations had to be carried out over the relatively long period of 180 min for optimal binding to occur. In contrast, IgG binding to lymphocytes in control experiments was maximal within 30 min. This relatively slow equilibration time for IgM may reflect the fact that, on freshly cultured lymphocytes, IgM receptors are occupied by endogenously bound molecules which must dissociate before binding can occur.

The apparent K_a of 2.5 X 10^9 M^{-1} for the high avidity interaction indicates that IgM binds more strongly to lymphocytes than does mouse IgG 2a to macrophages (K_a = 1.3 X 10^8 M^{-1}) (14); in fact, this interaction is nearly as strong as the binding of IgE to its receptor on mast cells (K_a = 1 X 10^{10} M^{-1}) (15). The intensity of the binding shown here, taken with the apparent localization of IgM receptors to T lymphocytes having helper cell activity (5), suggests that cell-bound IgM may play a biologically important role. Presumably receptor-bound IgM is in equilibrium with serum IgM proteins. Thus, at physiological

concentrations of IgM (1.6 μM) all of the high-avidity IgM binding sites should be saturated. Preud'homme *et al.* (16) have reported that naturally occurring 8S IgM is more effective than 19S IgM at inhibiting the formation of IgM-coated ox erythrocyte rosettes about lymphocytes. Thus, 8S IgM, perhaps derived by shedding of surface immunoglobulins from B lymphocytes, may normally occupy this receptor. Measurements of the association constants that describe the binding of these proteins to lymphocytes may lead to the identification of the protein that occupies lymphocyte IgM receptors *in vivo*.

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