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The IgA/IgM Receptor Expressed on a Murine B Cell Lymphoma Is Poly-Ig Receptor

Julia M. Phillips-Quagliata,* Samir Patel,† Jing-Kang Han,* Sergei Arakelov,* T. Dharma Rao,* Marc J. Shulman,‡ Shafie Fazel,§ Ronald B. Corley,‡ Margaret Everett,§ Michel H. Klein,* Brian J. Underdown,|| and Blaise Corthesy¶

T560, a mouse B lymphoma that originated in gut-associated lymphoid tissue, expresses receptors that bind dimeric IgA and IgM in a mutually inhibitory manner but have little affinity for monomeric IgA. Evidence presented in this paper indicates that the receptor is poly-Ig receptor (pIgR) known in humans and domestic cattle to bind both IgA and IgM. The evidence includes the demonstration that binding of IgM is J chain dependent, and that pIg-precipitated receptor has an appropriate Mr of 116–120 kDa and can be detected on immunoblots with specific rabbit anti-mouse pIgR. Overlapping RT-PCR performed using template mRNA from T560 cells and oligonucleotide primer pairs designed from the published sequence of mouse liver pIgR indicate that T560 cells express mRNA virtually identical with that of the epithelial cell pIgR throughout its external, transmembrane, and intracytoplasmic coding regions. Studies using mutant IgAs suggest that the Cα2 domain of dimeric IgA is not involved in high-affinity binding to the T560 pIgR. Inasmuch as this mouse B cell pIgR binds IgM better than IgA, it is similar to human pIgR and differs from rat, mouse, and rabbit epithelial cell pIgRs that bind IgA but not IgM. Possible explanations for this difference are discussed. All clones of T560 contain some cells that spontaneously secrete both IgG2a and IgA, but all of the IgA recoverable from the medium and from cell lysates is monomeric; it cannot be converted to secretory IgA by T560 cells. The Journal of Immunology, 2000, 165: 2544–2555.

Ig Fc receptors are of interest because of their ability to transduce signals between bound, cross-linked Igs and the cell interior, leading either to activation or down-regulation of cellular function (1). Because the predominant Ig in the gut-associated lymphoid tissue (GALT)3 is IgA, it is likely that specific receptors for IgA (FcαR) may participate in the regulation of GALT B cell behavior, but this is difficult to study because few lymphocytes express them (2–8). Several years ago we discovered a GALT-derived murine B lymphoma, T560, that expressed an IgA receptor (9). Thinking that it might regulate GALT B cell behavior, we studied its properties only to find that it differed from the classical FcαR in that its binding of IgA immune complex (IC) was inhibitable not only by IgA but also by IgM, implying that it was, in reality, an IgA/IgM receptor. IgA/IgM receptors were already well known as poly-Ig receptors (pIgR), responsible for transporting both dimeric and polymeric IgA (pIgA) and IgM (collectively referred to as plgs) through secretory epithelial cells into secretions (10–13). pIgR are not thought to be importantly expressed on lymphoid cells, but they have been detected on a T cell hybridoma that also expresses FcαR (14), and the ability of Abs to secretory component (SC), the portion of plgR bound to IgA in secretory IgA (S-IgA), to block binding of IgA to FcαR on GALT lymphoid cells of mice has been attributed to cross-reactivity between the FcαR and the plgR (15).

In this paper, we show that the T560 IgA/IgM receptor is plgR based on the following evidence: its binding of IgM is inhibited by plgA but not by S-IgA and is J chain dependent as is binding of plg by human (16, 17) and mouse (18) plgRs; it binds IgM through a different structure from the distinct IgM receptor (FcμR) of mouse T cells (19); it is precipitable with either IgA or IgM; its Mr (116 kDa) is consistent with its being plgR; and it is recognized by Abs to mouse plgRs on immunoblots. Furthermore, complete mRNA for plgR is contained in T560 cells. Studies of the binding properties of mutant mouse IgAs also presented in this paper indicate that the Cα2 domain probably does not mediate interaction between dimeric IgA and the mouse plgR. Because a small proportion of T560 cells coexpress IgA with IgG2a (20, 21) and the cell supernatant contains low concentrations of IgA, we considered the possibility that binding and uptake of autochthonous IgA might enable T560 to produce S-IgA. However, all of the IgA in both lysates and secretions of T560 cells proved to be monomeric; none of it had a high molecular mass consistent with that of S-IgA. T560 evidently does not convert endogenous IgA into S-IgA.

Materials and Methods

Cell lines

The T560.2.F7 and CH12.LX B lymphoma and the J774 macrophage cell lines were maintained as previously described (9, 22).

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3 Abbreviations used in this paper: GALT, gut-associated lymphoid tissue; FcαR, IgA receptor; FcμR, IgM receptor; IC, immune complex; MF, mean fluorescence; plgR, poly-Ig receptor; plg, polymeric Ig; plgA, polymeric IgA; PI-PLC, phosphatidylinositol-specific phospholipase C; SC, secretory component; S-IgA, secretory IgA; TNP, 2,4,6 trinitrophenyl; TNP-ORBC, TNP-ox RBC; wt, wild type; PKC, protein kinase C; CDR, complementarity determining region; RFC, rosette-forming cells; DPBS, Dulbecco’s PBS.

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Antibodies

All goat Abs and Ab conjugates were obtained from Southern Biotechnolog- 
Associate (Birmingham, AL). Rat mAbs to the following four mouse C\a domains, 
R3-23, rat IgG2a anti-C\a; B7/6, rat IgG1 anti-C\a; IM41, 
at IgG1 anti-C\a; and C2-23, rat IgG2 anti-C\a (19), were kindly pro-
vided by Dr. Richard Lynch (Department of Pathology, University of Iowa 
College of Medicine, Iowa City, IA). Rabbit anti-recombinant mouse plgR 
and denatured plgR and SC in Western blots has been described previously. 

The mouse anti-rat \k-chain mAb RGT9.1 (24) was purified from 
culture supernatants on protein A beads.

Detection of IgA and IgM binding by flow cytometry

Binding of IgA and IgM for detection by flow cytometry was done as 
described in Ref 9 except that binding was at room temperature rather than 
37°C. Various concentrations of IgA, IgM, or IgA-2,4,6 trinitrophenyl 
(TNP)-BSA IC (see below) or, for inhibition assays, mixtures of IgA 
and IgM, IgM and IgA, or IgM and anti-IgM mAb were used. Maximal 
binding of IgA or IgM was observed on T560 cells passed 24 h previously 
at 2 × 10^5 cells/ml, incubated at 37°C for 2 h in fresh medium, then 
was washed three times with Dulbecco’s PBS (DPBS) at room temperature 
followed by buffer appropriate to the assay. Binding of mouse IgM was 
detected with FITC-goat anti-mouse \k-chain or, when only MOPC-104E 
IgM was used, with FITC-goat anti-mouse \l-chain light chain; that of rat IgM 
was detected with FITC-goat anti-\k-chain, and that of human IgA was detected 
with FITC-goat anti-human \k-chain.

For inhibition studies, the percentage of inhibition was determined from 
the mean fluorescence (MF) in the various samples using the following 
formula: % inhibition = 100 (1 – (MF in inhibited sample – MF in low 
control))/MF (MF in uninhibited sample – MF in low control).

Immunoglobulins

Human IgA. Polymeric myeloma IgA (IgA polymer 1) and monomeric 
myeloma IgA from a single individual (Steele) were purified in the lab-
oratory of one of us (B.J.U.). Polymeric myeloma IgA (IgA polymer 2) 
and dimeric myeloma IgA from two different individuals, McElhany and 
Latimer, respectively, as well as normal human S-IgA, were kindly given 
to us by Dr. J. Mestecky (University of Alabama, Birmingham, AL).

Mouse IgA. BALB/c mouse myeloma IgA, TEP-15 (T15; IgA\k) and 
MOPC-315 (M315; IgA\k) were prepared as previously described (9, 21) or 
purchased from Sigma-Aldrich. Mutant M315 IgA\k myeloma proteins 
were prepared by cassette mutagenesis, changing Cys^{575}_k to Ala, Tyr^{302}_k to 
Phc, and Cys^{311}_k to Ala, and substituting the flexible human IgA\k hinge 
or the very short human IgA hinge 2 hinge for the mouse hinge region. Briefly, the 
pSV2-V_{\k} 315 plasmid was constructed by inserting an Xho-I fragment of 
J_{\k} 1-R\alpha and J_{\k} 30 segment together with an enhancer-containing Nov- 
EcoRI fragment of a JH3-JH4 probe into the Xhol and EcoRI sites of the 
multiple cloning site in pSV2NeoM (25). The wild-type (wt) pSV2-V_{\k} 315 plasmid 
was created by inserting a 1.5-kb Xhol-SalI fragment containing the 
BALB/c germline C\k gene derived from the C\k 30 phase clone (26) 
into the corresponding site of pSV2-V_{\k} 315. pUC-C\k 30 was made by 
excision of the C\k 30 fragment from pSV2-V_{\k} 315 and cloning it into a modified pUC18 with 
no EcoRI and one Xhol site. IgA mutant constructs were generated from pUC-C\k 30. The region encompassing 
residues 297–314 of wt C\k 30 is flanked by EcoRI and EcoRVIII restriction 
sites, which facilitated insertion of synthetic oligonucleotides containing 
the desired mutations. The mutants were sequenced to ensure a 
correct reading frame, and the mutant C\k was excised from pUC with 
Xhol and SalI and inserted into the pSV2-V_{\k} 315 plasmid. The wt and mutant 
IgA constructs were electroporated into cells of the light-chain-expressing 
recipient myeloma line M315.26. Mutant and wt IgAs in the supernatants 
of the transfected cells were used to coat TNP-ox RBC (TNP-ORB or 
for rosette assays (9). ELISA and agglutination titers were used to adjust 
the IgA concentrations to approximate those in our standard preparation of 
M315 IgA\k myeloma protein.

Mouse IgM. MOPC-104E (IgM\k) and TEPC-183 (IgM\k) were obtained from 
Sigma-Aldrich. TNP-specific normal and mutant IgMs were prepared from 
the supernatants of transfected cell lines expressing a TNP-specific 
\k-chain together with a normal or mutant TNP-specific \k-chain as previously 
described (27–29). SP-6 IgM has a wt \k-chain and consists mainly of 
monomers with some pentamers and tetramers lacking J chain, whereas 
J_{\k} 1-IgM-S414 (28) consists of polymers with J chain. J_{\k} 1-IgM-S575 has 
\k-chains with Ser substituted for Cys^{575}_k and consists mainly of monomers 
with some polymers lacking J chain. These IgMs were isolated from the 
monomers by binding to DNP-Sepharose followed by elution with 8 mM 
DNP-glucine. The eluates were dialyzed against PBS to remove the hapten 
and concentrated by ultrafiltration. A total of 200 ml of the concentrated 
SP-6, J_{\k} 1-IgM-S414, and J_{\k} 1-IgM-S575 protein solutions were ultraconcent-
trated on sucrose density gradients, essentially as described (27). Fractions 
(0.5 ml) collected from the top down were serially diluted and examined by 
ELISA, and the concentrations of IgM were determined (Fig. 1). Pools of 
“monomer” (fractions 4–8) and “polymer” (fractions 12–20) were concen-
trated by ultrafiltration and dialyzed against DPBS to remove sucrose, and 
their IgM concentrations were determined in ELISA. Insufficient SP-6 
monomer was obtained for experiments so only the polymer was used. The 
J_{\k} 1-IgM-S414 was concentrated but not subjected to sucrose gradient frac-
tionation. Mouse IgM was also isolated from supernatants of WEHI-231, 
which lacks J chain and makes mainly hexamer IgM, and from 
and, after 
washing the beads, eluting the IgM with 0.2 M methyl-\alpha-\alpha-mannopyrano-
side (Sigma-Aldrich). The eluates were concentrated by ultrafiltration, di-
alyzed against DPBS, and the IgM concentrations were determined by 
ELISA (see below) and adjusted to working concentrations by dilution 
in DPBS.

Other IgAs

Mouse IgG2Ax (UPC-10), IgG2Bx (MOPC-141; Sigma-Aldrich), and rat 
IgM (Rockland, Gilbertville, PA) were taken to have the concentrations 
specified by the manufacturer. Normal Sprague Dawley rat-IgG was 
preserved by binding to and elution from protein G beads (Pierce, Rockford, 
IL). Its concentration was determined from its OD_{280}.

Other proteins

Mouse whey was kindly given to us by Dr. Michael Lamm (Department of 
Pathology, Case Western Reserve University, Cleveland, OH).

Phosphatidylinositol-specific phospholipase C (PI-PLC) 

T560.2.F7 cells were incubated at 37°C with 50 ml PI-PLC from Bacillus 
subtilis (Boehringer Mannheim, Indianapolis, IN) and from Bacillus 
subtilis (ICN Pharmaceuticals, Costa Mesa, CA) in medium or in 
medium alone for 45 min in the presence or absence of 0.2 ng/ml calphos-
tin-C (Calbiochem-Novabiochem, La Jolla, CA), washed three times in 
medium containing FCS, and resuspended in DPBS for rosette assay as 
previously described (9).

ELISA

ELISA were performed as described in Ref. 21 using plates sensitized with 
anti-goat-mouse \k-chain or anti-mouse- or human \k-chain in PBS (pH 7.0). 
The concentrations of the various mouse IgM and IgA and human plgA 
preparations were determined by interpolation on standard curves. Because 
polymeric IgM and IgA titrate in ELISAs with different slopes from their 
monomeric counterparts, both the samples and the standards, MOPC-104E 
for mouse IgM, M315 for mouse IgA (both from Sigma-Aldrich), were 
reduced to monomers and alkylated before titration. Briefly, 0.1-m1 volumes 
of IgM or IgA in PBS containing 1 mg/ml BSA were mixed with 0.3

![FIGURE 1. Sucrose gradient separation of mutant IgMs J_{\k} 1-IgM-S414, 
J_{\k} 1-IgM-S575, and wt SP-6 IgM. IgM fractions 4–8 and 12–20 were pooled as 
“monomer” and “polymer.” respectively.](image-url)
ml 0.1 M Tris-HCl buffer (pH 8.6). DTT (0.1 ml, 0.03 M in the same buffer) was added, and the mixtures were incubated for 1 h at 37°C. After reduction, iodoacetamide (0.1 ml, 0.07 M in buffer) was added, and the mixtures were again incubated for 20 min at 37°C. Sodium acetate buffer (0.4 ml, 0.1 M) was then added to bring the pH to 7.0 and the volume to 1.0 ml. Serial dilutions of the standards and samples were applied to the sensitized ELISA wells. The human pIgAs had titration curves similar to that of the standard (human colostral IgA from Sigma-Aldrich) and so were not reduced, but the monomeric human IgA preparation had a titration curve of very different slope so its concentration was calculated simply from its OD. The concentration of rat IgM (Rockland) was taken as that specified by the manufacturer.

To check that the ability of the rat anti-mouse Cμ domain mAbs to bind to mouse IgM was intact, ELISA wells coated with 10 μg/ml of MOPC-104E IgM were blocked with BSA, and serial dilutions of the mAbs or of normal rat IgG were added to the wells and incubated. After washing, rat IgG bound to the wells was detected with HRP-goat anti-rat IgG Ab. All normal rat IgG were added to the wells and incubated. After washing, rat IgM and M315 IgA at 1 mg/ml were treated with an equal volume of 0.05% hydrogen peroxide in H2O were then added five times over the next 30 min, and the mixture was allowed to stand 10 min after the last addition. The volume was then brought to 10 ml with DPBS, and the cells were spun down and washed four times with DPBS. Aliquots of 2 × 107 T560 cells in 0.2 ml DPBS were lysed with 1 ml DPBS-based lysis buffer (pH 6.0) containing 0.5% Nonidet P-40, 0.01% soybean trypsin inhibitor, 10 μg/ml leupeptin, 1 μg/ml pepstatin A, 2 μg/ml chymostatin, 2 μg/ml antipain, 100 μg/ml PMSF, 10 mM benzamidine hydrochloride, 50 mM e-amino-caproic acid, 20 mM iodoacetamide, and 0.02% sodium azide (32) at 4°C for 1 h. After centrifugation, the supernatants were transferred to clean tubes and supplemented with 1/10 volume of 3.5 M NaCl containing 100 μg/ml PMSF and 3 mg/ml protease-free BSA. The supernatants were precleared by rotating them at 4°C overnight with mixtures of 30 μl each of packed plain protein G beads, protein G beads coated with goat anti-mouse IgM (for CH12 cells), IgA, and IgG and protein G beads coated with the eventual Ab to be used to bind IgM or IgA to the beads to precipitate the receptor (i.e., goat anti-mouse light chain for MOPC-104E or M315, and anti-rat κ-chain mAb RGT7.9.1 (24) for rat IgM). These beads were removed by centrifugation, and a second preclearance was performed by transferring the supernatants into fresh tubes containing 30 μl of packed MOPC-21 (mouse IgG1)-coated beads, rotating the mixtures at 4°C for 2 h. Equal volumes of the supernatants were then placed into fresh microfuge tubes containing 30 μl washed protein G beads precoated with either monoclonal anti-rat κ-chain followed by rat IgM or control, BSA-containing buffer, or goat anti-mouse light chain Ab followed by either M315 dimeric IgA, MOPC-104E IgM, or control, BSA-containing buffer. The lysates were rotated with the beads overnight at 4°C. The beads were recovered by centrifugation and washed twice with 1 ml high-salt lysis buffer, five times with 0.5 ml of this same buffer, and once with 1 ml low-salt lysis buffer. The protein was solubilized from the beads by boiling with 50 μl fresh Laemmli sample buffer (33) with or without 0.7 M 2-ME, and electrophoresed on SDS-PAGE. In some experiments, precipitation was done in ELISA plates instead of on beads (32). For preclearance, the wells were coated with goat anti-mouse light chain or TNP-BSA, washed with PBS, and blocked with BSA. The lysates were precleared seven times by serial transfer to and incubation at 4°C in such coated wells, then placed in wells coated with goat anti-light chain, followed by either MOPC-104E IgM, control BSA-containing buffer, or with TNP-BSA followed by M315 dimeric IgA or BSA-containing buffer. After adsorption to the coated wells, the lysates were removed, and the wells were washed seven times with PBS. Bound proteins were removed from the wells with Laemmli sample buffer with or without 0.7 M 2-ME and boiled for 5 min before separation on SDS-PAGE.

Immunoblotting

For immunoblotting, lysates or 10-fold concentrated supernatants of unlabelled cells were precleared with protein G beads precoated with goat anti-mouse κ-chain (T560 cell line) or mouse λ-chain Ab (CH12 cells), and the pIgR or IgA present were precipitated onto protein G beads coated as above with RT7.9.1 anti-rat κ-chain rat IgM IC, with goat anti-human λ light chain human IgA polymer (1) complex, with the Abs alone with

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Table 1. PCR primers used for sequencing the pIgR of T560

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2546 B CELL POLY-Ig RECEPTOR
BSA (controls), or with goat anti-α-chain Ab. For the plgR, 10% SDS-PAGE was used, and the proteins were electrophoretically transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA). For S-IgA, 5% SDS-PAGE was used, and the proteins were transferred to ζ-probe (Bio-Rad). The membranes were blocked with 10% BSA in PBS containing 0.05% Tween 20 and 0.02% sodium azide, washed in PBS-Tween 20 without azide, and stained with either rabbit anti-denatured recombinant plgR (23) or normal rabbit serum followed by HRP-conjugated goat anti-rabbit IgG heavy and light chains (human/mouse adsorbed) or HRP-conjugated goat anti-mouse α-chain, diluted in 5% normal goat serum/PBS-Tween 20. The bands were developed with ECL Western blotting detection reagents (Amersham, Arlington Heights, IL).

**PCR**

PCR were performed using plgR primers with cDNA templates prepared from T560.2.F7 cells, from mouse and rat liver, and from control J774 macrophages and CH12.LX B lymphoma cells, and the PCR products were TA-cloned and sequenced as previously described (21). Sequential, overlapping PCRs were done using one primer designed from the sequence contained within the preceding amplified fragment and a second primer designed from the mouse liver plgR sequence (34) 5′ or 3′ of the existing fragment. Combination of the sequences of all these fragments allowed determination of the complete sequence of the T560 plgR except for the 5′ and 3′ ends, which were given by the mouse liver primers. The names and locations of the primer sequences are given in Table I.

**Results**

**Cross-inhibition of binding of IgA and IgM to T560.2.F7 cells**

Rosette assays were previously used to show that T560 cells bind both mouse IgA- and IgM-coated erythrocytes and that these two IgGs are mutually inhibitory (9). Similar binding and inhibitory phenomena are here demonstrated by flow cytometry with both mouse and human IgA and mouse and rat IgM. Binding of polymeric M315 mouse IgA myeloma protein to T560.2.F7 cells in the presence or absence of TNP-BSA is shown in Fig. 2A. IgA binds to T560.2.F7 cells, and the presence of TNP-BSA in Ab excess markedly enhances the signal from FITC anti-α-chain Ab, presumably because there is more IgA to take it up in the bound complex. Not all of this IgA is necessarily in contact with receptors on the cell surface, but multipoint attachment to the cell surface receptors probably increases the avidity of binding of complexes over that of plgR alone. No binding to CH12.LX cells, which lack IgA/IgM receptors, is seen (Fig. 2B).

Inhibition of binding of M315 IgA-TNP-BSA IC to T560.2.F7 cells by normal rat IgM is illustrated in Fig. 3. More than 50% inhibition of binding of IgA complex containing 25 µg/ml IgA is achieved with as little as 9 µg/ml of rat IgM.

Binding of 40 µg/ml of MOPC-104E mouse IgM to T560.2.F7 cells is inhibited by human polymeric and dimeric IgA, but not at all by S-IgA and very little by monomeric IgA. Fifty percent inhibition requires ~100 µg/ml of IgA polymer (1) and dimer, but 350 µg/ml of IgA polymer (2) and >1000 µg/ml IgA monomer (Fig. 4). Because the inhibition curves for polymer and monomer have the same slope, it is possible that the inhibition by monomer is due to minor contamination by polymer.

We previously noted (9) that high concentrations of both mouse IgG2a (UPC-10) and IgG2b (MOPC-141) inhibited IgA rosette formation. These two proteins were shown not to be appreciably contaminated with IgA. In the present work, we tested the same lots of protein for their ability to inhibit binding of MOPC-104E IgM to T560.2.F7 cells as measured by flow cytometry. We found that IgG2a inhibited IgM binding by 40% at 300 µg/ml and by 16% at 150 µg/ml whereas, at the same concentrations, IgG2b enhanced it (data not shown). ELISA subsequently revealed that the IgG2b preparation was contaminated by IgM, which would

**FIGURE 2.** Binding of 50 µg M315 IgA myeloma protein/ml and of M315 IgA IC preformed in Ab excess (equal volumes of 100 µg/ml M315 IgA plus 12.8 µg/ml TNP27-BSA mixed and incubated for 1 h at room temperature) to T560.2.F7 (A) and CH12.LX (B) B lymphoma cells. Staining was done with FITC-goat anti-mouse α-chain. Dark line, IgA; light line, control. The numbers represent the MF. The MF of the FITC-goat anti-mouse α-chain control binding to T560.2.F7 cells was 15.0 and to CH12.LX was 25.6.

**FIGURE 3.** Inhibition of binding of M315 IgA IC preformed in Ab excess (equal volumes of 100 µg/ml M315 IgA plus 12.8 µg/ml TNP27-BSA were mixed and incubated for 1 h at room temperature) to T560.2.F7 cells by normal rat IgM. The IC were mixed with equal volumes of medium alone or of medium containing different concentrations of IgM to achieve the stated IgM concentration and 25 µg IgA/ml. Staining was done with FITC-goat anti-mouse α-chain. Dark line, IgA; light line, control. The numbers represent the MF with the percentage of inhibition in parentheses. The control MF was 13.2.

**FIGURE 4.** Inhibition of binding of MOPC-104E IgM myeloma protein at 40 µg/ml (final concentration) by human monomeric, dimeric, polymeric, and secretory IgA. This figure shows means of two to three experiments with each IgA, not all done at the same time. The concentrations have been corrected to those determined by ELISA.
explain why it inhibited IgA rosette formation in our earlier experiments. However, the inhibition of both IgA and IgM-binding by IgG2a is still unexplained for it was not contaminated by either IgA or IgM. Inhibition of IgM binding by IgG2a in the experiments discussed here is much less impressive than the 100% inhibition of IgA rosette formation seen with only 65 μg/ml IgG2a in our earlier study (9).

**Binding of IgA and IgM to the IgA/IgM receptor is not due to recognition of a shared carbohydrate**

To examine the possibility that IgA and IgM bind to the IgA/IgM receptor through a shared carbohydrate, the effect of periodate oxidation of the carbohydrate on binding of rat IgM and M315 IgA was tested. The data (Table II) indicate that treatment with periodate has little or no effect on the ability of IgM to bind to the cells (in the first experiment it reduced it, in the second it did not) but, in three experiments, it increased the ability of IgA to do so. It is not clear why it has this effect on IgA but not IgM. One possibility is that cleavage of sugar rings in the carbohydrates on IgA renders the IgA susceptible to aggregation, another is that it relieves a partial blockade of binding of IgA to the pIgR normally mediated by native carbohydrates. Whatever the explanation, there being no major reduction in binding of either protein, it seems unlikely that interaction with the T560 receptor is mediated through a carbohydrate shared between IgM and IgA.

**Binding of murine IgM to the T560 IgA/IgM receptor does not involve the same epitopes as binding to the IgM receptor on murine T cells**

Marked inhibition of binding of murine IgM to the T cell IgM receptor was shown to occur when either of two different rat mAbs (IM41 and 2911) to the Cμ3 domain were complexed with the IgM at ratios higher than 5:1 (Ab to IgM) before addition to the cells (19). Borderline inhibition was seen with one of two mAbs to the Cμ2 domain and no inhibition was seen with anti-Cμ1 or with either of two mAbs to the Cμ4 domain. We tested the ability of some of these same Abs to inhibit binding of MOPC-104E to the T560 IgA/IgM receptor at Ab: IgM ratios ranging from 8:1 down to 1:1. There was no inhibition of binding with any of the mAbs to any of domains Cμ1 through Cμ4, most notably not with IM41, which reacts with Cμ3, indicating that the epitopes bound by the mAbs are not involved in binding to the T560 IgA/IgM receptor (data not shown). Marked potentiation of the signal provided by FITC-goat anti-mouse λ light chain actually occurred with Abs to Cμ2 and Cμ3 but not with Abs to Cμ1 and Cμ4, suggesting that the former promoted the build-up of MOPC-104E-Ab IC on the cell surface.

**Role of J chain in the binding of IgM to the IgA/IgM receptor**

The best described IgA/IgM receptor to date is the pIgR, normally expressed on secretory epithelial cells. Expression of the structure recognized by this receptor is dependent on the presence of J chain in the IgA or IgM polymer (16–18). To examine a possible relationship between the T560 IgA/IgM receptor and the pIgR, binding of IgMs containing or lacking J chain was investigated. TNP-specific SP-6 mutant and wt IgMs were affinity purified from the supernatants of transfected cells, separated into monomers and polymers on sucrose gradients (J+/IgM-S414, J-IgM-S575, and wt SP-6) or left unseparated, (J+/IgM-S414), and their ability to bind to T560.2.F7 cells was assessed by flow cytometry (Fig. 5, A and B).

The data indicated that neither the polymers nor the monomers from the J− mutant IgMs could bind significantly to T560.2.F7 cells (there was a trace of binding of J+/IgM-S414 polymer) but J+/IgM-S414 and wt SP-6 polymeric IgM as well as additional control, unseparated IgMs, MOPC-104E, and TEPC-183 all bound very well. They suggested that plgM-lacking J chain does not bind to the receptor. However, they were not conclusive because the mutations in the μ-chains of S414 and S575 might have affected their ability to bind independently of affecting their J chain content.

The role of J chain in binding to the T560.2.F7 IgA/IgM receptor was further examined by comparing the binding of WEHI-231 IgM with normal μ-chains but lacking J chain (30) with that of IgM from two transfected cell lines: one, 3-C10, expressing J chain; the other, 3-H1, not expressing J chain. (Fig. 6). The results were clear cut. 3-C10 IgM-containing J chain, bound well to T560.2.F7 cells; WEHI-231 IgM and 3-HI IgM, both lacking J chain, failed to bind. Because WEHI-231 IgM is substantially hexameric, the results support the conclusion that it is not sufficient for IgM to be polymeric; it must contain J chain to bind to the receptor. The results are consistent with the idea that the T560 IgA/IgM receptor is a form of pIgR, known to require J chain for binding of polymeric Ig.

**Precipitation with plg identifies a 116-kDa receptor for IgM/IgM on T560 cells**

Radioiodinated IgA/IgM receptor was precipitated with plg from T560 cells under several sets of conditions. Both rat and mouse IgM precipitated it more readily than plgA. No similar molecule

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Protein</th>
<th>Staining Reagent</th>
<th>Periodate Treatment</th>
<th>MF</th>
</tr>
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<tbody>
<tr>
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<tr>
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<td></td>
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<td></td>
<td>IgA</td>
<td>FITC-goat anti-mouse α-chain</td>
<td>+</td>
<td>63.2</td>
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was precipitated from CH12 cells. In our earliest studies (20) using the protease inhibitors described in Ref. 14, triethanolamine, iodoacetamide, chymostatin, leupeptin, pepstatin, antipain, and PMSF, we precipitated small amounts of a molecule similar in size (36 kDa) to that of the IgA-binding 38-kDa molecule precipitated from murine T cells (35). However, when we added soybean trypsin inhibitor, aprotinin, diisopropylfluorophosphate, and sodium azide to the inhibitors mentioned above (32), we brought down a band of much higher Mr (116 kDa; Fig. 7), and the 36-kDa band disappeared. It seems likely that the 36-kDa band represented an IgM-binding product of proteolysis derived from the 116-kDa band. The apparent Mr of the 116-kDa band increased to about 120 kDa on 7.5% polyacrylamide gels but was unaltered under reducing vs nonreducing conditions (not shown). The band was also precipitated by IgA or IgM in PBS indicating that its binding is not dependent on divalent cation (not shown).

**Immunodetection reveals the identity of the 116-kDa protein as pIgR**

The receptor precipitated from Nonidet P-40 lysates of T560 cells with either rat IgM or human plgA was detectable as a band of ~116 kDa on immunoblots with rabbit Ab to denatured mouse pIgR followed by HRP-goat Ab to rabbit IgG (Fig. 8). No such band was precipitated from lysates of CH12 cells, which lack the IgA/IgM receptor. Control lanes show both free SC (~90–95 kDa) was precipitated from CH12 cells. In our earliest studies (20) using the protease inhibitors described in Ref. 14, triethanolamine, iodoacetamide, chymostatin, leupeptin, pepstatin, antipain, and PMSF, we precipitated small amounts of a molecule similar in size (36 kDa) to that of the IgA-binding 38-kDa molecule precipitated from murine T cells (35). However, when we added soybean trypsin inhibitor, aprotinin, diisopropylfluorophosphate, and sodium azide to the inhibitors mentioned above (32), we brought down a band of much higher Mr, (116 kDa; Fig. 7), and the 36-kDa band disappeared. It seems likely that the 36-kDa band represented an IgM-binding product of proteolysis derived from the 116-kDa band. The apparent Mr of the 116-kDa band increased to about 120 kDa on 7.5% polyacrylamide gels but was unaltered under reducing vs nonreducing conditions (not shown). The band was also precipitated by IgA or IgM in PBS in the presence of EDTA indicating that its binding is not dependent on divalent cation (not shown).
FIGURE 8. Immunoblot showing precipitation of 116-kDa band from T560 cells by rat IgM or human plgA on protein G beads. The beads were preincubated with RG7.9.1 anti-rat κ-chain, washed, and then incubated with rat IgM (M), diluent alone (MC), goat anti-human λ light chain followed by human IgA polymer (1A), or diluent alone (AC). Reduced samples were run on 10% SDS-PAGE, blotted onto nitrocellulose, and developed by anti-plgR Ab. Negative controls were done with CH12.LX cell lane stained with anti-plgR (shown), as well as with T560 cells stained with normal rabbit serum followed by HRP goat anti-rabbit IgG (not shown). Nonreduced (NR) and reduced mouse whey provided positive controls for staining of intact S-IgA (the high M₉ bands above 208 kDa) and free SC (the bands above 84 kDa) by the anti-plgR Ab.

FIGURE 9. Photograph of agarose gel electrophoretic analysis of the products of PCRs done with sense and antisense primers derived from the first and second domain of mouse plgR and with sense and antisense primers for β₂-microglobulin as a control. Lane 1, Ladder (1-kb); lane 2, mouse liver cDNA; lane 3, rat liver cDNA; lane 4, 3774 macrophage cell line cDNA; lane 5, T560.2.F7 cDNA; lane 6, CH12.LX lymphoma cell line cDNA; lane 7, T560.2.F7 cDNA (different preparation from that shown in lane 5); lane 8, no template control.

T560 cells express mRNA-encoding plgR

The binding characteristics of the IgA/IgM receptor, especially its requirement for J chain, its high molecular mass, and its ability to bind anti-murine plgR Ab suggest that it is plgR. The possibility that it might represent some new B cell form of the plgR, perhaps with GPI linkage to the cell membrane, as suggested by the sensitivity of the T560 IgA receptor to PI-PLC (9, 36), was next explored by RT-PCR. First we demonstrated (Fig. 9) that T560 cells contain message for the plgR using primers encompassing ~80% of domain I and a small portion of domain II of the mouse liver plgR sequence. Identically sized fragments (337 bp) were amplified from rat and mouse liver and from both of two separately prepared T560.2.F7 mRNAs but not from mRNA of either CH12.LX, the B cell line used as control above, or J774, a receptor-negative macrophage line (9), and not from a control containing no template. The fragments were TA-cloned and sequenced; fragments amplified from rat mRNA contained rat sequence while those amplified from mouse liver and from T560 contained mouse sequence.

Multiple, serial RT-PCRs were then performed using T560 mRNA as template and overlapping sets of primers. The fragments were TA-cloned and sequenced. The whole T560 plgR sequence is shown in Fig. 10. T560 cells clearly contain mRNA coding for a protein identical with epithelial cell plgR throughout its external, transmembrane, and intracytoplasmic regions except for a single T to C base change at position 476 that results in a Val→Ala change in domain II. This change, which could well be allelic, was verified by sequencing the product of an independent RT-PCR. Two C to T changes, one conservative in domain 4, the other in the 3’ untranslated region, have not been so verified. The sequence provided no grounds for supposing that the form of plgR on T560 was different from that on epithelial cells.

The sensitivity of the plgR on T560 cells to PI-PLC is due to activation of protein kinase C (PKC)

We previously noted (9) that treatment of T560 cells with PI-PLC destroyed their ability to bind IgA-coated erythrocytes, suggesting that the IgA receptor might be GPI-linked to the cell membrane. We also found that activation of protein kinase C (PKC) by PMA caused down-regulation of IgA receptor activity. Subsequently, we discovered (36) that the effect of PI-PLC was partially reversed by staurosporine, a protein kinase inhibitor. We now show that it is completely reversed by calphostin C, which is PKC-specific. As shown in Table III, treating T560 cells with PI-PLC reduces their ability to form IgA rosettes by ~85%. Addition of calphostin C diminishes the reduction due to PI-PLC to only 3%. These results suggest that PI-PLC causes loss of IgA receptor activity (by inference plgR receptor activity) from T560 cells indirectly, by activating PKC. PKC activation may follow cleavage by PI-PLC of a bona fide GPI-linked molecule from the cell surface (or much less likely) by PI-PLC itself crossing the cell membrane and cleaving phosphatidylinositols bisphosphate. Whatever the mechanism of PI-PLC-induced activation of PKC, it is clear that the T560 plgR is not GPI-linked to the cell membrane but has conventional type I transmembrane and cytoplasmic regions consistent with the sequence data presented above.

Lack of effect of mutation in the Ca₂ domain or hinge regions on binding of IgA to the plgR

Motifs in the Ca₂ domain have been postulated to mediate binding between IgA and the plgR. To explore the roles of Ca₂ domain Cys residues in the binding of IgA to the plgR, rosette assays were performed comparing normal and mutant IgA proteins. The residues targeted were Cys³⁰¹, its adjacent Tyr³⁰², and Cys³¹¹. Both Cys³⁰¹ and Cys³¹¹ are responsible for inter-α-chain bonding
within the IgA monomer. In addition, Cys 311 of one α-chain in an IgA dimer undergoes a disulfide exchange reaction and binds to a highly conserved Cys in pIgR domain 5 (Cys 467 in human pIgR) during the formation of S-IgA. At similar concentrations and agglutination titers, there was no significant difference between mutant and nonmutant IgAs in their ability to mediate rosette formation (Table IV). Discrepancies between the concentrations and agglutination titers probably reflect the ratios of monomer to polymer in the different supernatants. Because either of two Cys and a Tyr in the Cα2 domain can undergo change without impairing the ability of the dimeric IgA molecule to bind to the pIgR, the Cα2 domain is probably not directly involved in high-affinity binding to the pIgR. It also makes no difference to rosette formation whether the extended, flexible human IgA1 or the very short human IgA2 hinge is substituted for the mouse hinge region.

The IgA secreted by T56O cells upon switching from IgG2a to IgA is all monomeric and is not taken up and resecreted as S-IgA. Because T560 cells can produce IgA and contain mRNA for J chain as determined by RT-PCR (not shown), we explored the possibility that T560 might make dimeric IgA that could bind to the pIgR and then be internalized, processed in endosomes, and released as S-IgA. In immunoblots (Fig. 11), the lanes containing the supernatants and lysates of T560.2.F7 lanes contain α-chain bands with an Mr of ~100 kDa, consistent with α-chain dimers (the light chains of mouse IgA are not covalently bound to heavy chains). No tetrameric or higher molecular mass α-chain bands, such as are seen in the lanes containing T15 IgA, M315 IgA, and S-IgA (in mouse whey), are detectable.
Table III. Effect of calphostin C on PI-PLC-induced reduction in IgA rosette formation by T560.2.F7 cells incubated with M315 IgA-coated TNP-ORBC

<table>
<thead>
<tr>
<th>Supernatant Used to Coat</th>
<th>Mutation</th>
<th>Initial IgA Conc. (μg/ml)</th>
<th>Aggl. Titer</th>
<th>% IgA RFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>M315 standard (1/12)</td>
<td>None</td>
<td>123</td>
<td>1:32</td>
<td>45.4</td>
</tr>
<tr>
<td>FCS control</td>
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<td>0</td>
<td>0</td>
<td>2.4</td>
</tr>
<tr>
<td>Co30 (wt IgA) (1/2)</td>
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<td>15</td>
<td>1:4</td>
<td>31.3</td>
</tr>
<tr>
<td>1905 (1/2)</td>
<td>Cys801→Ala</td>
<td>35</td>
<td>1:32</td>
<td>47.1</td>
</tr>
<tr>
<td>2077 (1/2)</td>
<td>Tyr302→Phe</td>
<td>32</td>
<td>1:8</td>
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</tr>
<tr>
<td>1920 (1/2)</td>
<td>Cys811→Ala</td>
<td>126</td>
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<td>Human IgA1 hinge</td>
<td>14</td>
<td>1:4</td>
<td>36.9</td>
</tr>
<tr>
<td>IgA2 (1/2)</td>
<td>Human IgA2 hinge</td>
<td>272</td>
<td>1:64</td>
<td>51.3</td>
</tr>
</tbody>
</table>

a Means of duplicate samples. A minimum of 200 cells per sample was counted in either the RFC or non-RFC category.
FIGURE 11. Immunoblot showing IgA from the T15 and M315 IgA myelomas from mouse whey and from lysates and supernatants of T560.2.7F cells. The cells were grown for 3 days to a density of 1.6 × 10^6/ml. The cells contained in 35 ml culture fluid were centrifuged out and lysed with 0.5% Nonidet P-40. The supernatants were concentrated 10-fold by ultrafiltration. Both the lysates and supernatants were then adsorbed to protein G beads coated with goat anti-mouse α-chain Ab. After washing, bound IgA was eluted from the beads with Laemli sample buffer without reducing agent, run on 5% SDS-PAGE, and blotted onto 7-probe membranes. The membranes were stained with HRP-goat anti-mouse α-chain Ab. High molecular mass bands in the mouse whey lane represent murine S-IgA.

Complementarity determining region (CDR)1-, CDR2-, and CDR3-like loop (see Fig. 10) that may form a binding surface, much as they would in a conventional Ig (52). The CDR1-like loop of human plgR is essential for binding human IgA (50, 53, 54), but a distinct binding site containing the CDR2-like loop is critical for binding IgM (55). The amino acid sequences of mouse and rat plgR CDR-2 loops are identical but have Asn in place of the human Cys involved in interchain disulfide bonding were mutated to Ala. Of particular relevance, mutation of Cys in one of the pIgA subunits and pIgR domain 5 with Cys2 of the second subunit (57). It has been suggested (50) that initial binding of plgA to pIgR domain 1 may be followed by less avid interactions between the remaining plgR domains and pIgA that result in conformational changes and closer alignment of plgR with pIgA. Late in transcytosis of plgA-plgR complexes, a covalent bond is formed between a highly conserved Cys residue in plgR domain 5 and Cys in the Ca2 domain in one of the pIgA heavy chains (58, 59). Obviously, for this to happen these two domains must somehow be brought close together. That they have an affinity for one another that contributes to the overall affinity of binding is not supported, at least for the mouse system, by our data; it made no significant difference to IgA rosette formation whether either of the two Ca2 Cys involved in interchain disulfide bonding were mutated to Ala. Of particular relevance, mutation of Cys, which is actually used in disulfide bridging to SC, had no effect. This does not, of course, argue against the idea that a linear epitope of Cys2 independent of interchain S-S bonding might be involved in initial binding of dimeric IgA to pIgR domain 1.

The requirement for J chain for binding of IgM to the pIgR has been considered. T560, a B lymphoma, expresses plgR at all is a novelty. It raises the question whether plgR expression occurs naturally on a small cohort of normal B cells, of which T560 is a transformed
representative, or whether it is due to some aspect of the transformation process. Support for the notion that pIgR may be expressed on normal or activated lymphocytes comes from work showing that the splenocytes of mice carrying an IgA-secretory myeloma express IgA receptors whose ability to bind IgA is blocked by rabbit anti-rat SC (15). No other evidence indicating expression of pIgR by normal lymphoid cells has appeared, although numerous immunohistochemical studies have shown binding of anti-SC Abs to human intestinal epithelial cells in health and disease (65). If binding of anti-SC Abs to mucosal lymphocytes were common, it should surely have been recorded. Perhaps expression of the pIgR on the T560 lymphoma initially occurred as a response to cyto- kines available during malignant transformation. T560 is highly activated (22) with heavily mutated Ig heavy and light chains (21) and originated in the GALT of an F1 hybrid mouse that had been injected with parental splenocytes. Although signs of an ongoing graft-vs-host reaction were not apparent at the time of sacrifice, a graft-vs-host reaction had probably been initiated and then abrogated. Graft-vs-host reactions involve release of many cytokines and originated in the GALT of an F1 hybrid mouse that had been switched on in T560 as a consequence of translocation.

Medical Branch, Galveston, TX), is that the pIgR gene, normally present on the chromosomes of T560 and do not know the genetic mechanism of its expression. Support for the notion that pIgR may be expressed on B cells is that the pIgR gene, normally present on the chromosome of T560, is expressed on the T560 lymphoma as a consequence of translocation and originated in the GALT of an F1 hybrid mouse that had been switched on in T560 as a consequence of translocation.


