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Naturally Occurring IgM Anti-Leukocyte Autoantibodies (IgM-ALA) Inhibit T Cell Activation and Chemotaxis

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The physiological relevance of naturally occurring IgM-ALA remains to be elucidated. These autoantibodies are present from birth and increase in diverse inflammatory states that are both infectious and noninfectious. Clinical observations showing significantly less acute allograft rejections in recipients having high IgM-ALA levels, led us to investigate whether IgM-ALA could have a functional role in attenuating T cell mediated inflammatory responses. In pursuit of this hypothesis, we did studies using IgM purified from the serum of normal individuals, patients with end stage renal disease, and HIV-1 infection. All preparations of IgM immunoprecipitated certain receptors e.g., CD3, CD4, CCR5, and CXCR4 from whole cell lysates but failed to immunoprecipitate IL-2R and HLA Ags. In physiological doses IgM down-regulated CD4, CD2 and CD86 but not CD8 and CD28, inhibited T cell proliferation, decreased production of certain proinflammatory cytokines e.g., TNF-α, IL-13 and IL-2, but not IFN-γ, IL-1β, GM-CSF, IL-6 and IL-8 and inhibited leukocyte chemotaxis. These inhibitory effects were more pronounced when using IgM from patients with high levels of IgM-ALA and these inhibitory effects were significantly reduced after using IgM preabsorbed with leukocytes. IgM-ALA binding to leukocytes was found to be highly specific, as <10% of IgM secreting B cell clones had IgM-ALA specificity with some clones having specificity for either T cells or monocytes. These findings support the concept that IgM-ALA provides an innate mechanism to regulate T cell mediated inflammatory responses. The Journal of Immunology, 2008, 180: 1780–1791.

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immunoprecipitate CD3, CD4, CCR5, and CXCR4 (but not HLA and CD25) from whole cell lysates of lymphoid cell lines. There was however, a marked individual variation in levels of IgM with specificity to a single receptor (as determined by Western blots) and this variation was present in normals as well as disease states. The data show that polyclonal preparations of IgM with high binding reactivity to CD3, CD4 and chemokine receptors inhibits T cell activation and proliferation in response to alloantigens and anti-CD3 (OKT3) as well as inhibits binding of chemokines to their receptors.

Materials and Methods
Detection of IgM anti-lymphocyte Abs in cadaveric renal transplant recipients

Briefly, in this assay Ficoll-Hypaque isolated mono-nuclear cells are initially digested with pronase to remove receptors that bind to the Fc domain of Abs (e.g., CD16 that binds to Fc of IgG) and then reacted with recipients sera for 30 min at 4°C, washed two times at 4°C, and then interacted with FITC-labeled goat anti-human IgM (Fc specific) and PE anti-CD3 before quantitating IgM binding to T and non-T cells with two-color flow cytometry. IgM was centrifuged at the end of 45 min to express most of the leukocyte membrane receptors including CD3, CD4, and chemokine receptors. The IgM was centrifuged at the end of 45 min to remove cells, and the absorbed IgM was quantitated using ELISA techniques. In the absorption technique, 50–60% of IgM was lost. Absorbed IgM had <15% residual binding activity to Jurkat cells, U937 cells, lymphocytes, neutrophils, or cultured endothelial cells as determined by flow cytometry. Absorbed IgM was re-run through the column (Sepharcl S-300 HR) to remove cytokines that may have accompanied the large number of leukocytes.

Preparation of monomeric IgM

Monomeric IgM was made from the pentameric form in 200 nM Tris, 150 mM NaCl, and 1 mM EDTA (pH 8.0), and by reduction with 5 mM DTTO for 2 h at room temperature. Subsequent alkalization was performed for 1 h on ice with 12 mM sodium phosphate. IgM monomers were isolated from any remaining pentameric forms by column chromatography. Supernatant (Supermax 200) equilibrated with PBS. Purity of monomeric IgM was confirmed with SDS-PAGE Western blots under reducing and nonreducing conditions. With flow cytometry, one observed <20% reduction in binding of monomeric IgM to lymphocytes when compared with the pentameric form.

Cell Lines

All the cell lines were obtained from the American Type Culture Collection Program at National Institutes of Health. SupT-1 and Jurkat cell lines are human CD4 and CD3 positive T cell leukemia lines constitutively expressing high levels of CXCR4. Hut78 is a human T cell line that secretes IL-2 and constitutively expresses IL-2R, CD-8, CCR5 and CXCR4. U937 is a human monocytoid leukemia monocyte cell line constitutively expressing CD4, CXCR4, CCR5, CCR4, CCR3, CCR2B and CCR1, but not CD3. Daudi is a B cell lymphoma cell line expressing HLA-class II, CXCR4 and CCR5 but not CD3, HLA-class I and no membrane Ig.

Antibodies

Anti-CCR5 murine IgG monoclonals (clones 2D7, CTC-5, 45502, 45523, and 45549) and anti-CXCR4 (CTC-5, 12G5, 44708, 44717, and 44716) were obtained from R&D Systems. Clone 4G10, a murine IgG monoclonal that binds to the N-terminal region of CXCR4 was a gift from Dr. Chris Broder (33). The following Abs were used as primary Abs in the Western blot procedure: polyclonal rabbit IgG Abs to CD3 (Santa Cruz Biotechnology), CD4 (R&D Systems), or CXCR4 (Bioschain); monoclonal mouse IgG Abs to CCR5 (clone CTC, N-terminal) and CXCR4 (clone 4G10 N-terminal).

Immunoprecipitation technique with purified IgM and Western blot procedure

Cell lines (80 × 10^6) were incubated for 30 min at 4°C with 10 ml of 100 mM (NH₄)₂SO₄, 20 mM Tris-HCl (pH 7.5) containing 10% glycerol, 1% Cymal-5 (Anatrace) and 1 tab minicomplete (Roche) to solubilize membrane receptors with minimal denaturation (34). Because different cell lines vary in their expression of the different receptors, we used lysates of SupT-1 and Jurkat cells to immunoprecipitate CD4, CD3, and CXCR4. Daudi B cells for CXCR4, which is mostly nonglycosylated, Daudi and U937 cells for CCR5. IgM/receptor complexes were formed by interacting 100 μl of recombinant soluble CD4, recombinant soluble CXCR4 and 20% reduction in binding of monomeric IgM to lymphocytes when compared with the pentameric form.
CD4 protein is glycosylated and comprises the full length of the extracellular domain (aa 1–370).

**Human IgM secreting B cell clones**

B cells were obtained from human umbilical cord blood by initially isolating the leukocytes with histopaque and then subjecting the cells to the B cell negative isolation magnetic bead kit (Dynal Biotech). Purified B cells (95% pure) were dispensed at 5,000 cells per well (96-well plate) in RPMI 1640 containing 10% FCS. 50,000 irradiated (3000 rad) human PBL were added to each well as feeder cells. B cells were then infected with EBV containing supernatant from cell line B95–8 (American Type Culture Collection). After 5 wk in culture, culture supernatants were examined for IgM using an ELISA technique. Ninety percent of wells had IgM detected by the ELISA technique, with ~18% of culture wells at concentrations of 300–1000 ng/ml (high IgM). Each of these high IgM containing supernatants were examined for IgM-ALA with a pool of leukocyte cell lines i.e., Jurkat, SupT-1, Daudi, and U937. Similarly, supernatants were examined for IgG and IgM anti-CD4 reactivity using an ELISA technique (see below). Of note, the wells at this stage did not have monoclonal IgM secreting B cell clones.

**ELISA to detect IgM or IgG binding to CD4**

We used a previously described technique (35); 100 µL containing 250 ng of recombinant, full length extracellular domain of CD4 (Progenics) were added to a 96-well nunc-immulon plate and the ELISA plate was prepared using the Immuno-Tek ELISA construction system (Zephto Metrix). A total of 200–300 ng of IgM from serum, culture supernatants, or purified IgM was added to the wells and incubated at room temperature for 1 h. The secondary Ab used was a 1/4000 dilution of HRP goat anti-human IgM or IgG (Southern Biotechnology Associates).

**MLR assay**

In these studies, we used Ficoll-Hypaque isolated PBMC (PBL). Briefly, 0.15 × 10⁸ PBL in 0.15 ml RPMI 1640 containing 10% FCS were cocultured (in triplicate) with similar number of cells from another individual in 5% CO₂ before adding cells from the second individual.

**PBL activation and proliferation with anti-CD3 or PHA plus IL-2**

OKT3 (0.01–1.0 µg/ml) or PHA (5 µg/ml) plus IL-2 (40 U/ml) was added to Ficoll-Hypaque isolated PBL (1 × 10⁶ cells/ml) in RPMI 1640 and 10% FCS. Cells were cultured in triplicate in a flat-bottom BD Falcon 96-well tissue culture plates. After 5–6 days in culture, [³H]Thymidine was added to cells in each well, and cells were harvested 12–18 h later. Different doses (5–30 µg) of IgM were added to each well on day 0 and day 1 of the culture period. For day 0 cocultures, cells from one individual was initially incubated with IgM at 37°C for 30 min in 5% CO₂ before adding cells from the second individual.

**Quantitation of cytokines**

Cytokines in PBL culture supernatants were assayed either by an ELISA technique or in a semiquantitative manner using the Ray Bio human cytokine array no. 3 kit (Ray Biotech), which consists of a membrane array containing 42 different murine cytokines. Each array was blocked with PBS containing 5% FCS and in 5% CO₂ for 1 h containing supernatant from cell line B95–8 (American Type Culture Collection). After 5 wk in culture, culture supernatants were examined for IgM using an ELISA technique. Ninety percent of wells had IgM detected by the ELISA technique, with ~18% of culture wells at concentrations of 300–1000 ng/ml (high IgM). Each of these high IgM containing supernatants were examined for IgM-ALA with a pool of leukocyte cell lines i.e., Jurkat, SupT-1, Daudi, and U937. Similarly, supernatants were examined for IgG and IgM anti-CD4 reactivity using an ELISA technique (see below). Of note, the wells at this stage did not have monoclonal IgM secreting B cell clones.

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**MLR assay**

In these studies, we used Ficoll-Hypaque isolated PBMC (PBL). Briefly, 0.15 × 10⁸ PBL in 0.15 ml RPMI 1640 containing 10% FCS were cocultured (in triplicate) with similar number of cells from another individual known to have different HLA-class-1 and DR Ags. Cells were cocultured in flat-bottom BD Falcon 96-well tissue culture plates. After 5–6 days in culture, [³H]Thymidine was added to cells in each well, and cells were harvested 12–18 h later. Different doses (5–30 µg) of IgM were added to each well on day 0 and day 1 of the culture period. For day 0 cocultures, cells from one individual was initially incubated with IgM at 37°C for 30 min in 5% CO₂ before adding cells from the second individual.

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OKT3 (0.01–1.0 µg/ml) or PHA (5 µg/ml) plus IL-2 (40 U/ml) was added to Ficoll-Hypaque isolated PBL (1 × 10⁶ cells/ml) in RPMI 1640 and 10% FCS. Cells were cultured in triplicate in a flat-bottom BD Falcon 96-well plate (0.3 × 10⁶ cells) for 2–4 days at 37°C in 5% CO₂. Cytokine arrays were quantitated as in the MLR assay. The effect of IgM was evaluated by initially pretreating cells with IgM at 37°C for 30 min in 5% CO₂ before adding OKT3.

**Quantitation of chemokine binding to cells**

Cells (0.5 × 10⁶ in 0.5 ml) obtained from T cell lines (Hut 78 and Jurkat E-6) or monocytoid cell line U937 (937) or PBL activated for 3 days with PHA plus IL-2 were incubated with or without IgM (1–30 µg/ml, 10⁶ cells/ml) in PBS buffer containing CaCl₂ at 37°C for 45 min, and without a wash step, cells were re-incubated at 37°C for 45 min with biotin-labeled cytokine (50 ng). Cells were then washed in the cold and stained with PE-streptavidin and chemokine binding was quantitated by flow cytometry.

**CXCL12 (SDF-1α) induced down-regulation of CXCR4**

Cells (0.2 × 10⁶/0.2 ml) expressing CXCR4 were pretreated with medium (PBS containing CaCl₂ and 2% FCS) or medium with 3 µg of normal IgM (pre-absorbed with mouse IgG) at 37°C for 30 min, not washed and then cells were incubated with (25–100 µg) at 37°C for 30 min. Cells were then washed at 4°C and incubated with FITC-labeled anti-CXCR4 (12G5, murine IgG mAb) at 4°C for 45 min.

**Authorization for human subjects**

The human investigation committee (HIC) at our institution has approved this study and informed consent was obtained from all subjects.

**Results**

High IgM-ALA levels are associated with minimal acute rejections and better graft survival

Before a human kidney transplant, sera from the recipient is routinely reacted with the potential donor’s lymphocytes to exclude the presence of IgG anti-HLA Abs, which can give rise to hyperacute or accelerated rejection, and to determine whether the recipient has IgM reactive to donor lymphocytes. Table I depicts the incidence of biopsy proven acute rejections in kidney allograft recipients having different quantities of IgM-ALA reactive to their donor lymphocytes. None of these recipients were empirically treated for a rejection without a biopsy. Recipients with high levels...
of IgM anti-donor lymphocyte (IgM-ALA) reactivity had significantly less rejection episodes when compared with the other subset of patients. All recipients were on similar anti-rejection medications, i.e., prednisone, cyclosporin A or Prograf and Mycophenolate (Cell Cept). These differences in rejection rates among the three groups of recipients did not correlate with age, sex, race, level of HLA Ag mismatch and propensity to develop anti-HLA Abs pretransplant.

These data, together with previously published data, would indicate that IgM-ALA may have a role in inhibiting or attenuating the inflammatory response mediated by acute rejections (27–31). Presence of high IgM-ALA did not correlate with age, sex, race, renal disease causing ESRD or pretransplant exposure to CMV and EBV viruses. Similarly, there was no difference in total IgM serum levels among the three groups of recipients. These IgM anti-lymphocyte autoantibodies had reactivity to T, B, and NK cells, and also CD14 positive macrophages and CD15 positive neutrophils (data not shown). Prior studies also revealed reactivity of IgM to endothelial cells obtained from human kidneys and the myeloid K562 cell line lacking HLA class I and II receptors (36).

These in vivo observations of decreased rejections and the in vitro finding that IgM bound to T cells and other leukocytes and endothelial cells prompted us to examine whether IgM mediated these beneficial effects by inhibiting T cell activation and proliferation as well as by inhibiting the inflammatory process mediated by chemokines and chemokine receptors. Binding of IgM to chemokine receptors appeared to be an attractive possibility and could explain the widespread binding of IgM to the different leukocyte and endothelial cells.

**Purified IgM has more IgM-ALA activity than serum IgM**

Certain normal, ESRD or HIV sera have minimal or no IgM-ALA reactivity when evaluated by IgM binding to leukocytes (Table I) or to immobilized recombinant soluble CD4. In these studies, soluble CD4 was also used to evaluate IgM-ALA activity as IgM immuno precipitated CD4 from whole cell lysates as well as soluble recombinant CD4 (data presented in Fig. 3). We therefore wanted to determine whether purified IgM, isolated from such sera, had similarly low IgM-ALA activity. Surprisingly, purified IgM, obtained from sera with low IgM-ALA activity, had substantial more IgM binding to leukocytes and immobilized CD4, i.e., high IgM-ALA activity (Fig. 1, A and B). This avid binding to leukocytes and immobilized CD4 with purified IgM, was not observed with IgG purified from the same sera. Furthermore, purified IgM, when combined with autologous sera, maintained this enhanced IgM-ALA activity indicating that the observed lack of IgM-ALA activity with normal serum is not due to a serum factor that inhibits IgM binding (Fig. 1, A and B). However, absorbing purified IgM with leukocytes removed IgM-ALA activity (Fig. 1C). Taken together, these observations would suggest that the apparent lack of IgM-ALA reactivity in certain sera is a result of IgM-ALA binding to leukocyte receptors, which are then shed in an IgM/receptor complex in the serum. The IgM purification process dissociates IgM from the receptor complex thus enhancing IgM-ALA reactivity. Hence, presence of high IgM-ALA reactivity in certain sera, as exemplified for lymphocyte binding in Table I and for CD4 binding in Fig. 1B (see HIV no. 12, no. 50), can best be explained by increased IgM-ALA production, which tilts the balance in favor of more noncomplexed IgM-ALA in serum.

**Evidence to show specific binding of IgM-ALA to leukocyte receptors**

Because IgM is a pentameric molecule and is encoded by nonmutated germline genes, it became necessary to exclude the possibility of nonspecific binding by IgM to carbohydrate moieties on leukocyte receptors. This possibility was examined by evaluating the binding of IgM from a panel of IgM secreting human B cell clones derived from human umbilical cord blood. Of the 79 supernatants containing IgM (concentration, 300–1100 ng/ml), only 8 had IgM secreting clones with IgM-ALA activity. Clones secreting IgM-ALA had the following binding specificities to cell lines: 2 clones with only anti-T cell (Jurkat, SupT-1) reactivity, 2 clones with only anti-monocyte (U937) reactivity and 4 clones with reactivity to all cell lines i.e., T, B and U937 (monocytoid) cells (Fig. 2A). Two of these 8 wells also had IgM that bound to soluble CD4 as evaluated by an ELISA technique (Fig. 2B). All 8 clones secreting IgM-ALA did not bind to IgG coated latex beads (data not shown). These findings i.e., presence of IgM-ALA reactivity in 10% of IgM secreting clones (and not the majority of IgM secreting clones) and binding of IgM-ALA to CD4 and specific cell lines i.e., T cells or monocytes (and not the majority of IgM secreting clones) would strongly argue against nonspecific IgM binding to leukocyte receptors and provide in addition evidence indicating that IgM-ALA consists of different Abs having different specificities e.g., IgM with specificity for CD4 and another Ab with specificity for a receptor on T or monocyte cells. Furthermore, binding of IgM to all cell lines i.e., T cells, Daudi B cells and U937 can best be explained either by polyspecificity of certain IgM autoantibodies or by binding of IgM-ALA to a leukocyte receptor that is common to all cell lines e.g., CD45.

**Naturally occurring IgM-ALA are present at birth**

The production of IgM-ALA by umbilical cord B cells provided more evidence to show that IgM-ALA belong to the repertoire of naturally occurring IgM Abs. We therefore evaluated umbilical cord serum to determine the quantity of IgM using an ELISA technique (Zepto Metrix, NY) as well as to determine whether IgM-ALA was present in serum. Of the 7 sera analyzed, IgM was detectable in 6 sera at concentrations of 30–40 µg/ml while in one serum (cord blood no. 7) the IgM was present at 130 µg/ml. All cord sera had IgM-ALA (Fig. 2C). Such data indicate that IgM-ALA is present in normal sera at birth.

**Naturally occurring IgG-ALA are rare in sera and in supernatants of umbilical cord B cell clones**

Previous studies demonstrating the presence of naturally occurring IgG autoantibodies, with reactivity to intracellular proteins,
prompted studies to examine whether IgG-ALA are also present in newborn sera, normal sera and disease sera (37). In these studies, presence of IgG-ALA was evaluated by binding of IgG from B cell clone supernatants or sera to cell lines. No IgG-ALA was detected in 96 umbilical cord B cell clone supernatants, in 12 cord sera and in 27 normal adult sera. However, six of 135 ESRD sera had IgG-ALA that is specific for either T or non-T cells or all four cell lines. Subsequent studies revealed that IgG anti-non-T cell had binding reactivity to only U937 cells and not to Daudi cells. B, Data on IgG anti-CD4 reactivity using an ELISA technique. 50 μl supernatant containing 300 ng of IgG/ml was added to each well (see Materials and Methods). Note that only 2 of 79 IgG containing supernatants had IgG anti-CD4 reactivity and both these supernatants also had IgG with binding to leukocytes. C, IgG-ALA in cord blood serum. Serum (125 μl) from cord blood was added to a mixture of cells lines as in A. Note that cord serum no. 7 had IgG-ALA that bound to all four cell lines.

In the studies to be described below, we analyzed the effect of IgM-ALA on T cells and the chemokine receptor by using purified polyclonal IgM rather than serum and controlled for nonspecific IgM binding to leukocyte receptors by (i) using purified IgM obtained from different individual donors that included normals and patients with ESRD or HIV-1, and (ii) using IgM from a patient who was diagnosed with Waldenstrom’s macroglobulinaemia and had a monoclonal IgM with binding reactivity to an undefined leukocyte receptor. We could not identify any purified normal IgM as a negative control as all the purified normal IgM from normals had IgM-ALA reactivity.

FIGURE 1. IgM-ALA reactivity in purified IgM and autologous serum.
A. Representative data from five different IgM preparations, showing significantly more IgM binding to T cells with purified IgM than with autologous serum. Binding of purified IgM is not decreased when combined with autologous serum. In these experiments, sera or purified IgM containing 6 μg of IgM was added to 100,000 PBL preactivated with PHA plus IL-2. B. IgM anti-CD4 reactivities in individual purified IgM and corresponding autologous sera from normal (N) and HIV-1 patients using an ELISA technique (see Materials and Methods). Sera containing 300 ng of IgM was compared with 300 ng of purified IgM. Note that there is significantly more IgM anti-CD4 reactivity in purified IgM. Also note that some HIV sera, e.g., nos. 12 and 50, have high IgM anti-CD4. Absorbing purified IgM with leukocyte cell lines (C) removes IgM-ALA reactivity. 300 μg of purified normal IgM was absorbed x2, each time with a mixture of 250 x 10^6 cell lines (Jurkat, SupT-1, U937, Daudi and RAJI). Data are representative of four different IgM preparations.

FIGURE 2. Presence of IgM-ALA in supernatants from umbilical cord B cells and in cord blood serum. A and B, IgM-ALA reactivity in IgM containing supernatants from B cell clones activated with the EBV virus. Supernatants contained between 300 and 1000 ng of IgM/ml. A, IgM-ALA reactivity that was detectable in 8 of 79 supernatants. Here, 75 μl supernatants were incubated with 100,000 cells containing a mixture of cell lines, i.e., Jurkat, SupT-1, Daudi, and U937. Note that the supernatants have IgM-ALA that is specific for either T or non-T cells or all four cell lines. Subsequent studies revealed that IgM anti-non-T cell had binding reactivity to only U937 cells and not to Daudi cells. B, Data on IgM anti-CD4 reactivity using an ELISA technique. 50 μl supernatant containing 300 ng of IgM/ml was added to each well (see Materials and Methods). Note that only 2 of 79 IgM containing supernatants had IgM anti-CD4 reactivity and both these supernatants also had IgM with binding to leukocytes. C, IgM-ALA in cord blood serum. Serum (125 μl) from cord blood was added to a mixture of cells lines as in A. Note that cord serum no. 7 had IgM-ALA that bound to all four cell lines.
of these receptors were immunoprecipitated by HIV and ESRD IgM from whole cell lysates. Of note, IgM from several different normal, HIV, and ESRD individuals failed to immunoprecipitate IL-2-R, HLA-A, and HLA-DR receptors (data not shown). Interestingly, even though normal IgM immunoprecipitated low levels of CD4 from the cell lysates (see Fig. 3A), one clearly observed that the same dose of normal IgM immunoprecipitated significantly more CD4 when using the soluble recombinant CD4 (extracellular domain). Differences in CD4 conformation and epitope availability between recombinant and native CD4 as previously suggested, could explain these observations (38). However, binding of IgM to membrane expressed CD4 was ascertained by using B cell clones (e.g., 4G4) secreting IgM with reactivity to recombinant CD4 (ELISA). As can be seen from Fig. 3C, IgM from clone 4G4 had significant binding to CD4+ T cells when compared with CD4- T cells (MCF 71.5 vs 16.6). Similar data could not be obtained with polyclonal IgM as binding of the multiple different anti-receptor Abs present in the IgM preparations obscured the increased binding of IgM anti-CD4 to CD4+ T cells. Taken together, data from Figs. 1B, 2B, and 3A and C, clearly demonstrate that IgM binds to membrane expressed CD4 in a specific manner. Normal or ESRD IgM failed to immunoprecipitate chemokines i.e., recombinant CCL5 and CXCL12 (data not shown).

FIGURE 3. Immunoprecipitation experiments to show binding of IgM to CD3, CD4, CCR5, and CXCR4. Identical quantities of individual (labeled no. 1, 2, etc.) or pooled (labeled P) IgM from normal (labeled N), HIV (labeled H), ESRD (labeled E), or Waldenstrom (labeled W) were used to immunoprecipitate leukocyte receptors from equal amounts of whole cell lysates or recombinant soluble CD4. Jurkat and SupT-1 cell lysates were used for CD3/CD4, Daudi B cell lysates for CXCR4, and DaudiU937 lysates for CCR5. As controls, Western blots were performed with cell lysates in the absence of agarose beads (to control for binding of primary Ab to leukocyte receptor and to determine receptor size (labeled Ly). In another control, agarose beads without IgM were added to lysate to determine whether the leukocyte receptor nonspecifically bound to the bead (B plus Ly). Note that several fold more receptors were immunoprecipitated by ESRD and HIV IgM when compared with normal IgM (B). Also note that CXCR4 in Daudi is in a nonglycosylated form. IgM preferentially immunoprecipitated the monomeric form of CXCR4 or CCR5 receptor (as depicted in these figures) but certain normal and patient IgM also immunoprecipitated lower levels of the dimeric or other low m.w. isoforms of these receptors as previously described (34, 59) (data not shown). The primary polyclonal Ab detected all these receptor isoforms in the lysates we used. In A, recombinant, glycosylated, solubilized CD4 (Sol-CD4), comprising the full length extracellular domain was immunoprecipitated by IgM. For controls, Western blots were performed with soluble CD4 in absence of IgM and agarose beads (labeled CD4) and with Sol-CD4 added to agarose beads in absence of IgM (labeled CD4 B). C, Significantly increased binding of IgM from B cell clone 4G4 to CD4+ T cells when compared with CD4- T cells (MCF 71.6 vs 16.6)). Clone 4G4 secreted IgM with anti-CD4 reactivity detected by ELISA. Note that no increased binding was observed on CD4+ T cells using a B cell clone (IE12) secreting IgM without anti-CD4 reactivity (MCF 22.5 vs 22.3). Both clones have IgM anti-leukocyte binding. In these experiments 200,000 PBL were initially incubated (4°C) with identical quantities of IgM (1 μg), washed and then simultaneously stained with PE anti-CD3, FITC anti-CD4 and Cy5 anti-human IgM. These data are representative data obtained from two clones with anti-CD4 reactivity and 8 clones without anti-CD4 reactivity.

IgM inhibits T cell proliferation, cytokine production, and Zap-70 phosphorylation

Because IgM immunoprecipitated CD3 and CD4, it became important to determine whether purified IgM inhibited T cell activation, proliferation and cytokine production. Data in Fig. 4 indicate that purified IgM, especially ESRD IgM, inhibits T cell proliferation in response to alloantigens and anti-CD3 only when IgM was added to the cultures within the first 24 h of initiating the cultures. Certain individual normal IgM (e.g., N no. 1), unlike the other 5 normals, significantly inhibited the proliferative response in the allogeneic MLR (see Fig. 4B) indicating that the repertoire of IgM-ALA can vary in their functional activity, even among normals. However, in general, ESRD individual IgM was more inhibitory when compared with HIV IgM ALA. The pentameric form of IgM is not essential for the inhibitory effect on MLR as monomeric IgM was as effective (Fig. 4B). This inhibitory effect of ESRD IgM on proliferation was not associated with increased cell death or apoptosis as determined by flow cytometry using propidium and annexin reagents. The inhibitory effect of IgM-ALA on cell proliferation was not mediated by any contaminating IgG in the IgM preparation as pooled normal, ESRD, or HIV IgG failed to inhibit proliferation. The inhibitory effect of IgM-ALA on MLR-induced proliferation was most likely mediated by binding of IgM to CD3 (OKT3) and/or CD4 as additional experiments with IgG murine monoclonals clearly showed that Abs against CD3 or CD4 but not CXCR4 (12G5) and CCR5 (2D7) inhibited MLR-induced T cell proliferation (see Fig. 4B). Prior studies have shown that anti-CD3, even though mitogenic to T lymphocytes, will inhibit proliferation of T cells in an MLR (39).

Further studies were performed on supernatants from MLR cultures to determine whether the anti-proliferative effects of IgM-ALA were associated with a decrease in cytokine production. The Array III kit detected a significant increase in the basal secretion of IL-6, IL-8, IL-13, TNF-α, GM-CSF, MCP-1, MIG, MDC, TARC, and GRO when PBL were activated in an MLR assay. However, the addition of both normal and patient IgM at the initiation of the MLR culture, had no inhibitory effect on MLR-induced production of IL-6, IL-8, MCP-1, MIG, GRO, GM-CSF, IL-1β and TGFB-β but significantly decreased the quantity of certain other cytokines e.g., TNF-α, IL-13, MDC and TARC in the MLR supernatants (see Fig. 5A). This decrease in cytokine level (e.g., TNF-α) in the presence of IgM was not a result of IgM neutralizing the secreted TNF-α, as the addition of IgM directly to the control MLR supernatants, obtained on day 5, did not decrease the level of TNF-α.
detected by two assay systems (i.e., Array III and an ELISA technique), thus indicating that IgM specifically inhibits the secretion or production of certain cytokines. Because the Array III kit could not detect IL-2 and IFN-\(\gamma\)-\(\gamma\) in MLR supernatants, we resorted to determine the effect of IgM on intracellular expression of these cytokines. Both normal and ESRD IgM caused a major decrease in the percentage of cells expressing intracytoplasmic IL-2, but had no effect on IFN-\(\gamma\)-\(\gamma\) expressing cells (see Fig. 5B). Under the culture conditions, there was no detectable IL-17 or IL-10 in the supernatants or cytoplasm. Nonetheless, IgM did not increase production of these two cytokines. The combined data, with the different cytokine assay techniques, clearly demonstrate that normal and patient IgM predominantly inhibits the production of certain cytokines involved in T cell proliferation (e.g., IL-2) and certain proinflammatory cytokines and chemokines (e.g., TNF-\(\alpha\), IL-13, MDC and TARC). Conversely, IgM did not significantly inhibit other proinflammatory cytokines (e.g., IFN-\(\gamma\), IL-6, GM-CSF, IL-1\(\beta\) and IL-8).

Because prior studies have shown that TCR/CD3 receptor activation leads to increased intracellular phosphorylation of Zap-70, we evaluated whether IgM, after binding to CD3 and/or CD4, will inhibit anti-CD3 mediated phosphorylation of Zap-70. Phosphorylated Zap-70 and total Zap-70 were quantitated by flow cytometry at 0, 2, and 5 min and at 16 h after immobilized anti-CD3 activation of freshly isolated human PBL. IgM had a mild inhibitory effect on anti-CD3-induced phosphorylation of Zap-70 at 2 min. However, the majority of individual normal, ESRD and HIV IgM (but not Waldenstrom) inhibited both background phosphorylation of Zap-70 and the increase in Zap-70 phosphorylation induced by anti-CD3 at 16 h in 40–50% of T cells (see Fig. 5C). Importantly, IgM mediated inhibition of phos-Zap-70 was not associated with inhibition of total Zap-70 indicating that IgM inhibits proximal intracellular signaling (see Fig. 5D). Pooled normal IgG had no effect on Zap-70 phosphorylation (data not shown).

**Purified IgM down-regulate CD4 and CD2 but not naturally arising regulatory T cells**

The inhibitory effect of purified normal and ESRD IgM on T cell proliferation and TNF-\(\alpha\) production in response to alloantigen stimulation (MLR) prompted us to evaluate whether the inhibitory effects were also mediated by IgM down-regulation of receptors involved in T cell activation. In these studies, cell receptor expression was quantitated by flow cytometry using PBL activated either in an MLR or with PHA and IL-2. IgM was added to the cultures either at the initiation of the 4–5 day cultures or after T cells were activated. As exemplified in Fig. 6A, both normal and ESRD IgM, but not Waldenstrom IgM, when incubated for 4 h at 37°C with 5 day activated T lymphocytes, down-regulated CD2 and CD4 on T lymphocytes. Similar data were also obtained with HIV IgM (data not shown). Importantly, only ESRD and HIV IgM, but not normal IgM down-regulated CD86 (B7-2) on CD14 macrophages activated in an MLR for 24 h (Fig. 6C). However, normal and ESRD IgM did not change receptor expression (including up-regulation) of CD3, CD8, intracellular CD152, CD154, PD-1, CD28, CD40L, HLA-AB and HLA-DR on lymphocytes as well as CD80 (B7-1), CD40, and ICAM-1 on macrophages (data shown for CD8 and CD28 in Fig. 6A). Pooled normal IgG failed to downmodulate any of these receptors (data not shown).

Receptor down-regulation of CD2 and CD4 did not appear to be a result of receptor internalization as down-regulation of surface receptor expression was also accompanied by intracellular down-regulation of CD4 (see Fig. 6B). No down-regulation of CD2 and CD4 was observed by incubating MLR activated cells with IgM for 4–6 h at 4°C, indicating that the observed down-regulation of CD4 at 37°C was an active process and not due to IgM inhibiting the binding of the murine monoclonal used to detect CD4. Additionally, down-regulation of CD2 and CD4 occurred in the presence of monomeric IgM but the extent of down-regulation was ~50% when compared with pentameric IgM (data not shown).

Finally, we wanted to determine whether the inhibitory effect of IgM on T cell proliferation and cytokine production was secondary to IgM mediated increase in naturally arising regulatory T cells (i.e., CD4\(^+\), Foxp3\(^+\) cells expressing high levels of CD25). Representative Data in Fig. 6D show that ESRD IgM, when added at the initiation of an MLR culture, did not increase the CD4\(^+\) regulatory T cell subpopulation expressing high levels of CD25 and had no effect on total IL-2R levels and intracytoplasmic Foxp3 levels even though IgM significantly decreased CD4 receptors on T cells (MCF of CD4 changed from 429 to 79).

Thus far, the data indicate that purified polyclonal IgM from normals, HIV and ESRD patient’s immunoprecipitate CD3 and CD4, down modulate CD4 and CD2 and inhibit T cell activation (Zap-70) and proliferation. These inhibitory effects were more pronounced when using ESRD or HIV IgM. Furthermore ESRD IgM down modulated CD86, a costimulatory molecule, present on macrophages. Of particular interest, even though there was some variability in the functional effect of individual IgM e.g., on receptor down modulation or MLR, the different IgM preparations had similar functional effects i.e., predictably down modulating certain receptors e.g., CD4 and CD2 but not other receptors e.g., IL-2-R, HLA, CD8, CD3 and CD28. Similarly, the different IgM preparations...
had a similar effect in inhibiting only certain cytokines, particularly TNF-α and IL-2, and not other proinflammatory cytokines.

**Purified IgM inhibits chemokine binding, chemotaxis, and CXCL12-induced down-regulation of CXCR4**

Because IgM immunoprecipitated CXCR4 and CCR5 from cells (Fig. 3), it became important to determine whether purified IgM inhibited binding of chemokine to these receptors as well as inhibited chemotaxis. Data in Fig. 7A clearly demonstrate that both normal and ESRD IgM inhibited binding of CCL3 (MIP-1α) to CCR5 and CXCL12 to CXCR4 present on a cell line (U937) and on PBL activated for 3 days with PHA and IL-2. IgM inhibited chemokine binding in a dose-dependent manner as exemplified for binding of CCL3 to U937 cells and CXCL12 to activated PBL. Incubating cells with IgM and/or chemokine at 37°C or 4°C did not change the magnitude of the inhibitory effect of IgM on chemokine binding thus indicating that the IgM mediated inhibitory effect was not due to IgM-induced internalization of the receptor at 37°C. Furthermore, the lack of chemokine binding in the presence of IgM was not due to IgM forming a complex with the chemokine as we failed to detect IgM binding to the chemokine using immunoprecipitation techniques and an ELISA technique with immobilized chemokine. Waldenstrom IgM and pooled human IgG had no inhibitory effect on CXCR4 receptor. These differences in inhibitory effects on chemotaxis with the T cell lines were not due to increased apoptosis or cell death as evaluated by flow cytometry using propidium and annexin and would suggest that ESRD IgM in addition inhibits chemokinesis through effects on other cell receptors (e.g., adhesion molecules or integrins) and/or intracellular activation pathways that are involved in both chemokinesis and chemotaxis activity. Data in Fig. 7B show that both normal and ESRD IgM also has an inhibitory effect on chemokinesis of cells.

Because IgM inhibited binding of chemokine to their receptor, it became important to determine whether purified IgM also inhibited chemokine-induced receptor down-regulation as has previously been described (40). In these studies, cell lines expressing high levels of CXCR4 (Jurkat T cell, RAJI B cell) were used. Data in Fig. 7C clearly show that CXCL12 markedly down-regulates CXCR4 expression on the cell membrane secondary to receptor internalization. However, pretreatment of cells with IgM inhibits the CXCL12-induced down-regulation of CXCR4. Waldenstrom IgM had no inhibitory effect on CXCL12-induced down-regulation of CXCR4 (data not shown). Similar studies could not be done with cell lines expressing the CCR5 receptor as IgM inhibited binding of all the murine monoclonal anti-CCR5 Abs that were needed to detect membrane expression of CCR5.

These studies indicate that purified IgM binds to chemokine receptors and through this mechanism inhibits chemokine binding, chemokine-induced chemotaxis, and chemokine-induced receptor down-regulation. Finally, we failed to demonstrate binding of IgM to chemokines using both immunoprecipitation and ELISA techniques.
Discussion

The current studies clearly show that IgM-ALA are naturally occurring as these Abs are present in all umbilical cord serum (Fig. 2C) and umbilical cord B cell clones secrete IgM-ALA. Furthermore the evidence indicates that IgM-ALA binding to leukocyte receptors has specificity. First, several different individual purified IgM, whether from normal or diseased sera, bound to the same receptors, inhibited production of certain specific cytokines and down-regulated only certain cell receptors. Secondly, the data clearly show that IgM from certain individuals binds to CXCR4 but not CCR5 while IgM from other individuals bind to CCR5 and not CXCR4 (Fig. 3B). These latter findings strongly argue against nonspecific binding of IgM to membrane receptors. One would have expected purified IgM from different individuals as well as monoclonal Waldenstrom IgM to randomly bind to different receptors and to randomly down-regulate different receptors if there was nonspecific binding of polyreactive IgM to receptors. However, specificity of IgM binding is best exemplified by 1) analysis of IgM-ALA from B cell clone supernatants with 10% of IgM secreting clones having specificity for leukocytes, and with some IgM binding to either monocytes or T cells (Fig. 2A); 2) studies with purified IgM from individual HIV-1 sera clearly showing that IgM from certain individuals will only bind to CXCR4 and not CCR5 while IgM from other HIV individuals will bind to CCR5 and not CXCR4 (Fig. 3); and 3) lack of IgM-ALA binding to immobilized IgG.

The occasional presence of IgG-ALA in disease sera e.g., ESRD, but not in normal sera, and normal umbilical cord sera would argue against these Abs as being naturally occurring. IgG-ALA, unlike IgM-ALA, are cytolytic in the presence of complement (at 37°C) or Fcγ IgG receptor bearing cells (ADCC) (41). Hence there is a possibility that such autoantibodies are pathogenic (e.g., cause lymphopenia) and under normal conditions inhibitory mechanisms could prevent B cells from secreting IgG autoantibodies. Finally, it is unclear whether IgG-ALA are encoded by nonmutated germline genes.

There has been very little progress in understanding the biological role of naturally occurring IgM anti-leukocyte autoantibodies (IgM-ALA) even though these autoantibodies were first identified ∼35 years ago (13). A major difficulty has been the lack of information regarding the identity of the different leukocyte receptors which bind to these IgM-ALA. However clues on the potential identity of some of these receptors became apparent when we attempted to explain certain clinical observations in human kidney and heart transplants. Allograft recipients with high levels of IgM-ALA were found to have significantly better allograft survival and fewer rejections even during the precyclosporin era (27–31). Recognizing that rejection in an allograft is a T cell mediated inflammatory process, we hypothesized that IgM-ALA mediated these inhibitory effects by binding to leukocyte receptors involved in T cell activation and leukocyte chemotaxis. We felt that such an

FIGURE 6. Effect of purified IgM on costimulatory molecules and CD4⁺, CD25⁺, Foxp3⁺, regulatory T cells. A. Individual normal and ESRD IgM, but not murine IgG anti-CD3 (OKT3) inhibits CD2 and CD4 expression on PBL activated in MLR for 4 days. Data represent percent change in MCF in presence of Abs when compared with MCF in medium control. Day 4-activated cells were incubated for 16 h at 37°C in medium (control) or with ESRD or normal IgM, 10 μg/ml (obtained from a single individual) or soluble OKT3 (1 μg/ml). Note that IgM had a marked inhibitory effect on CD4 and CD2 receptors but not CD8 and CD28. Data are from eight separate experiments with different IgM and values represent mean ± SD. B. IgM inhibits both surface and intracytoplasmic CD4. Cells were initially labeled with PE anti-CD4 to quantitate surface CD4 and then permeabilized and relabeled with PE anti-CD4 to quantitate both surface and intracytoplasmic CD4. Normal IgM was added at initiation of MLR assay. Data in this figure is a representative example from one of five individuals. C. The effect of individual IgM on membrane CD86 expressed by CD14 positive macrophages present in PBL activated in a 24 h MLR assay. Note that HIV and ESRD IgM, but not normal IgM, inhibit CD86. D. The effect of IgM on CD4⁺ CD25(highly) Foxp3⁺ regulatory T cells in MLR-activated (day 5) PBL (upper panels). Pooled ESRD IgM (15 μg/ml) was added at initiation of MLR. There was no significant change in the percentage of this subpopulation or in intracytoplasmic Foxp3 in the presence of IgM. This is a representative example of three separate experiments with three different individual IgM. In all panels, MCF values are obtained from 10,000 gated events.
RAJI cells were either pretreated with CXCL12 (50 ng/ml) or purified normal IgM (300 ng/ml) and incubated with or without IgM (1–30 ng/ml) obtained from monocytic cell line (U937) or PBL activated for 3 days with PHA plus IL-2 were re-incubated at 37°C for 45 min with biotin-labeled cytokine (50 ng). Cells were then washed in the cold and stained with PE-streptavidin. These are representative examples from seven different experiments. In these experiments, MCF of CXCL12 binding to U937 was 193, whereas MCF of CCL3 binding to U937 was 600. MCF for negative controls was <10. ESRD IgM is more inhibitory to chemotaxis and chemokinesis when compared with normal IgM (B). Hut 78 or Jurkat cells were incubated with or without IgM (obtained from different individuals) at 37°C for 30 min before adding the cells, without a wash step, to the upper chamber of the chemotaxis Transwells. CXCL12 was added to the bottom wells. Data labeled media indicate baseline chemokinesis in the absence of CXCL12. Addition of CXCL12 to both the upper and lower wells completely inhibited chemotaxis (data not shown). These are representative examples from five separate experiments. Purified normal IgM (C) inhibits CXCL12-induced down-regulation of CXCR4. Details of assay are in Materials and Methods. 0.2 × 10^6 Jurkat and RAJI cells were either pretreated with CXCL12 (50 µg/ml) or initially with purified normal IgM (3 µg/ml) followed 30 min later with CXCL12 and then incubated at 37°C for 30 min before quantitating membrane CXCR4. In all panels, flow cytometry data were obtained from at least 10,000 events.

FIGURE 7. Effect of purified IgM on chemokine binding, chemotaxis, and CXCL12-induced CXCR4 down-regulation. A, Normal and ESRD IgM have a similar inhibitory effect on binding of biotin-labeled CXCL12 and CCL3 to cells. Cells (0.5 × 10^6 in 0.5 ml) obtained from monocytic cell line (U937) or PBL activated for 3 days with PHA plus IL-2 were incubated with or without IgM (1–30 µg/L × 10^6 cells/ml) in PBS buffer containing CaCl_2 at 37°C for 45 min, and without a wash step, cells were re-incubated at 37°C for 45 min with biotin-labeled cytokine (50 ng). Cells were then washed in the cold and stained with PE-streptavidin. These are representative examples from five separate experiments. Normal and ESRD IgM is more inhibitory to chemotaxis and chemokinesis when compared with normal sera (H9262 and H11003). 0.2 × 10^6 Jurkat and RAJI cells were either pretreated with CXCL12 (50 µg/ml) or initially with purified normal IgM followed 30 min later with CXCL12 and then incubated at 37°C for 30 min before quantitating membrane CXCR4. In all panels, flow cytometry data were obtained from at least 10,000 events.

hypothesis could also explain the increase in IgM-ALA in various inflammatory and infective states, perhaps to attenuate the inflammatory response (13–19).

Data in the present investigations show that IgM-ALA immuno-precipitates CD3, CD4, CXCR4 and CCR5 but not IL-2R and HLA. No attempt has been made in the current studies to elucidate the identity of the entire repertoire of leukocyte receptors that bind to IgM-ALA, which have been known to be heterogenous and to have binding reactivity to receptors of different molecular sizes, including glycoproteins, phospholipids and glycolipids (20–26). However, our data demonstrate that IgM-ALA 1) will inhibit T cell activation and proliferation in response to alloantigens and the mitogenic effect of anti-CD3; 2) down-regulates costimulatory molecules, e.g., CD2 and CD4 on T cells and CD86 on macrophages; and in addition 3) IgM-ALA inhibits chemotaxis induced by CXCL12 or CCL3. These observations support the concept that IgM-ALA could provide an innate mechanism to regulate a T cell mediated inflammatory response.

Data in Fig. 1, A and B, clearly demonstrate that purified IgM has substantially more IgM-ALA reactivity especially when compared with unpurified IgM present in autologous sera. A potential explanation for this observation is that most of the IgM-ALA in certain sera, especially normal sera, exists in an IgM/receptor complex which forms when the IgM binds to the receptor on the cell membrane. The purification process dissociates the IgM-ALA from the receptor complex. Support for such a concept comes from two observations 1) purified IgM obtained from normal sera or from sera with an apparent lack of IgM-ALA reactivity has high binding of IgM to leukocytes and this enhancement in IgM-ALA reactivity is not inhibited even when purified IgM is combined with autologous sera, and 2) IgM-ALA reactivity in purified IgM is removed when purified IgM is preabsorbed with leukocytes. Hence, the observed enhanced IgM-ALA reactivity of certain sera obtained from ESRD or HIV patients (see Table I and Fig. 1B) can best be explained on the basis of increased production of IgM with anti-leukocyte reactivity thus resulting in an increase in the level of uncomplexed IgM-ALA in such sera.

Interestingly, the data on T cell activation and proliferation indicate that purified IgM from normal, HIV and ESRD sera have similar effects on 1) CD2 and CD4 down-regulation; 2) inhibition of anti-CD3-induced phosphorylation of Zap-70; and 3) inhibition in production of IL-2 and certain proinflammatory cytokines (e.g., TNF-α) in response to alloantigen (MLR) stimulation. These observations raise two basic questions. Firstly, why does normal purified IgM down-regulate CD2 and CD4, inhibit Zap-70 phosphorylation and inhibit cytokine production? Secondly, how can one explain the pronounced inhibitory effects of ESRD and HIV IgM on MLR and anti-CD3-induced proliferation of T cells (see Fig. 4) considering that normal, HIV and ESRD IgM have a similar down regulating effect on CD2 and CD4 as well as on Zap-70 phosphorylation? A potential explanation centers on the physiological role of IgM-ALA. We postulate that under normal conditions, there is production of low levels of IgM-ALA primarily to attenuate T cell activation and the inflammatory response especially to autologous Ags and peptides. However, when confronted with excess T cell activation e.g., by nonself Ags or an acute or chronic inflammatory state, there is a significant increase in production of IgM-ALA and in addition there is recruitment and expansion of other B-1 cell clones that produce IgM with reactivity to other cell receptors as evidenced by ESRD (but not normal) IgM’s effect on CD86 (B7.2) to further regulate excess T cell activation, proliferation, and production of certain proinflammatory cytokines. In support of such a concept are observations in mice that are only deficient in IgM. Such mice exhibit an increased tendency to develop autoimmune diseases (42, 43). Additional support for such a conclusion also derives from studies of other investigators who clearly showed that impaired T cell activation and proliferation of T cells seen in ESRD patients were associated with a defect in macrophage activation of T cells. Macrophages from ESRD patients could not activate purified T lymphocytes from normal individuals and ESRD macrophages were found to have significantly decreased expression of CD86 (B7.2) (44).

Particularly intriguing is the observation that IgM-ALA down-regulates certain specific receptors (e.g., CD4 and CD2 on T cells and CD86 on macrophages) as well as certain cytokines (e.g., IL-2, TNF-α and IL-13). Perhaps such an innate mechanism evolved to inhibit receptors and cytokines that play a more dominant role in
certain inflammatory processes. In support of such a concept is the observation that an Ab to a single proinflammatory cytokine (i.e., anti-TNF-α) significantly improves the acute inflammatory manifestations in patients with rheumatoid arthritis or Crohn’s disease as well as the inflammatory response triggered by an allograft rejection in humans (45, 46).

Currently, our studies have not addressed the mechanism by which IgM-ALA down-regulates CD2, CD4 and CD86 receptors or how IgM-ALA attenuates T cell activation and proliferation. Several potential mechanisms may be operative. IgM, by binding to CD3 and CD4, could directly inhibit T cell activation in an MLR. Murine IgG monoclonals specific for CD3 and CD4 have been shown to inhibit activation and proliferation of human T cells in response to alloantigens (MLR) (39, 47). Secondly, IgM by inhibiting IL-2 production could also inhibit T cell proliferation. Finally, recent studies provide indirect evidence to show that chemokine receptors can also influence or inhibit intracellular signaling involved in T cell activation (48, 49).

It is therefore possible that IgM-ALA could also operate via such a mechanism. Clearly studies using monoclonal IgM-ALA with specificities for different receptors should help unravel some of the mechanisms leading to inhibition of T cell activation and proliferation in presence of IgM-ALA.

Several mechanisms could also be operative in IgM-ALA mediated inhibition of chemotaxis. An obvious mechanism is the inhibition of chemokine binding to the receptor. IgM also inhibited chemokinesis, which could result from IgM inactivating intracellular signaling or down-regulating receptors involved in chemokinesis and/or chemotaxis.

Finally, and of particular interest, is the observation that the repertoire of IgM-ALA can vary in normals as well as in patients. This is best exemplified with IgM-ALA obtained from HIV-I patients, where in certain patients IgM-ALA bound predominantly to CXCXR4 and minimally to CCR5 and vice-versa. Similarly IgM from a normal individual (labeled no. 1) had a marked anti-proliferative effect in the allogeneic MLR (Fig. 4B) even though the majority of other individual IgM obtained from normals, had no or minimal inhibitory effect on the MLR. In general, the majority of individual ESRD IgM was more inhibitory than HIV-I IgM in the various assay systems. Normal IgM, in general, was the least inhibitory. Such variability may have functional consequences. For example, it is possible that individuals with IgM-ALA that fails to inhibit the production of TNF-α could be predisposed to a more acute and serious inflammatory response after exposure to a foreign Ag.

The maintenance of immune system homeostasis is essential for protecting the host against excess inflammation and autoimmunity (50, 51). In this regard, T cells that have escaped central deletion or silencing mechanisms are subjected to peripheral control mechanisms. Homeostasis maintenance by different subsets of natural or silencing mechanisms are subjected to peripheral control mechanisms leading to inhibition of T cell activation and proliferation. Several potential mechanisms may be operative. IgM, by binding to CD3 and CD4, could directly inhibit T cell activation in an MLR. Murine IgG monoclonals specific for CD3 and CD4 have been shown to inhibit activation and proliferation of human T cells in response to alloantigens (MLR) (39, 47). Secondly, IgM by inhibiting IL-2 production could also inhibit T cell proliferation. Finally, recent studies provide indirect evidence to show that chemokine receptors can also influence or inhibit intracellular signaling involved in T cell activation (48, 49).

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The maintenance of immune system homeostasis is essential for protecting the host against excess inflammation and autoimmunity (50, 51). In this regard, T cells that have escaped central deletion or silencing mechanisms are subjected to peripheral control mechanisms. Homeostasis maintenance by different subsets of natural and adaptive regulatory T cells (CD4+ or CD8+), NK-T cells and dendritic cells have gained particular attention for understanding peripheral regulation of self reactive T cells (Fig. 8) (52–56). Other observations would also indicate that TH1 inhibitory cytokines and certain costimulatory molecules (e.g., CD152, PD1, and PD12) serve as additional peripheral control mechanisms that inhibit T cell mediated immune response (56–58). Our studies would suggest yet another innate immune regulatory mechanism that involves a noncytolytic (at 37°C) naturally occurring IgM anti-leukocyte autoantibody to maintain immune homeostasis and protect against autoimmunity (Fig. 8). Potential mechanisms for IgM-ALA include inhibition of Th cell activation with decreased production of certain specific proinflammatory cytokines, e.g., TNF-α, IL-2. Importantly the current data would also indicate that IgM-ALA controls the magnitude of the inflammatory response by not broadly inhibiting all proinflammatory cytokines (possibly to avert excess immunosuppression) and secondly by regulating leukocyte chemotaxis. Furthermore, it would appear that IgM-ALA may not inhibit the function of CD8+ T cells as CD8 is not down-modulated. Studies are in progress to determine whether IgM-ALA inhibits CD8+ regulator T cells and CTLs. Additionally, the marked individual variation in the repertoire of IgM with specificity to the different leukocyte receptors that we observed in both normal and disease states, may play a role in contributing to the differences in the vigor or character of the inflammatory response among different individuals when exposed to the same inciting agent.

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Disclosures

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