Inflammatory cells have surface receptors for immune complexes (1–12). These receptors play a functional role in phagocytosis (13) and cell-mediated cytotoxic reactions (14–21); and may be involved in immunoregulation (22, 23). The binding of IgG complexes to lymphocytes and macrophages does not require divalent cations (3, 7, 8). On the other hand, macrophage binding to IgM complexes has been shown to require Ca ++ (3). IgM complex receptors have also been detected on lymphocytes of several species (9–11). In this report we show that mouse lymphocytes exhibit optimal binding to IgM complexes in the presence of Mg ++ in contrast to macrophage IgM complex binding. In addition to their functional implications, these findings allow one to distinguish IgG from IgM binding and macrophage from lymphocyte IgM binding by their differential divalent cation requirements.

Materials and Methods

Lymphocyte suspensions were prepared from spleens, thymi, and lymph nodes of C3H/HeJ and CBA/Jax mice and used at a concentration of 10⁷ cells/ml in barbital-buffered saline (BBS), pH 7.2. Macrophages from thioglycollate-induced peritoneal exudates of outbred Swiss (CD-1) mice were cultured in monolayer on glass coverslips as previously described (13) and washed in BBS before use. Antisera to ox erythrocytes (ORBC) were collected 5 d after primary or tertiary immunization of CBA mice with 0.2 ml of 50% ORBC. The sera were fractionated by ammonium sulfate precipitation and Sepharose 6B (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) column chromatography; the leading half of the 19S peak from primary sera and the trailing half of the 7S peak from hyperimmune sera were collected for use. The 19S IgM fraction had a complement-dependent hemolytic titer of 256 and was used at a dilution of 1:10 in rosette assays. The 7S IgG, titer of 128, was used at a dilution of 1:50. 2% suspensions of ORBC sensitized with the appropriate antibodies were mixed with equal volumes of lymphocyte suspensions or were added to adherent macrophage cultures. Unsensitized erythrocytes served as controls. Various concentrations of Mg ++ or Ca ++ (chloride salts) or of EDTA or EGTA (sodium salts) were included as indicated below. After 15 min at 37°C the lymphoid cells were examined for rosette formation.
TABLE I

| Source         | 15 mM Mg** | 15 mM Ca** |
|----------------|------------|------------|
| Spleen§        | 22 ± 1.5   | 3 ± 0.5    |
| Lymph Node     | 11 ± 1.5   | 1 ± 0.3    |
| Thymus         | 9 ± 1.0    | 1 ± 0.2    |
| Peritoneal Macrophages§ | 4 ± 1.0  | 96 ± 1.5   |

* Divalent cation-free BBS did not support IgM-sensitized erythrocyte binding by either macrophages or lymphocytes.
§ Similar results were obtained from CBA, C3H, and CD-1 mice.
§§ Obtained from CD-1 mice.
¶ Data represents mean ± SEM of the percentage of rosette-forming cells from four experiments. Criteria for rosette formation were two or more erythrocytes attached to lymphocytes and three or more attached to macrophages.

Results

Spleen cells, thymocytes, lymph node cells, and macrophages were tested for their divalent cation requirement for optimal IgM complex binding. As shown in Table I, Mg** supported IgM complex binding by lymphocytes, whereas Ca** did not. The reverse was found with macrophages: Ca**, but not Mg**, supported IgM complex binding. Different concentrations of Mg** and Ca** (0–25 mM) were used to assess the concentration required for optimal IgM complex binding by spleen cells (Fig. 1). Increased IgM rosette formation was seen as the concentration of Mg** increased up to 20 mM. Ca** did not support lymphocyte binding to IgM complexes at any concentration; indeed, Ca** was inhibitory when added to the rosette-forming mixture that contained an optimal concentration of Mg** (data not shown).

The divalent cation requirement for IgM complex binding was further studied by inhibition with chelating agents. As shown in Table II, the addition of EDTA decreased IgM rosette formation by spleen cells at each dose tested; in contrast, 5 mM EGTA produced an increase in IgM rosette formation, and higher concentrations of EGTA were less inhibitory than comparable levels of EDTA. Table III shows that
Table II

Inhibition of IgM Complex Binding to Spleen Cells by Chelating Agents

| Chelating agent final concentration* | Percentage of control with 20 mM Mg++ |
|-------------------------------------|--------------------------------------|
|                                | EDTA | EGTA |
| mM                                 |      |      |
| 0‡                                 | 100§ | 100  |
| 5                                  | 27   | 136  |
| 10                                 | 18   | 66   |
| 15                                 | 14   | 39   |
| 20                                 | 14   | 39   |
| 25                                 | 9    | 36   |

* Chelating agents were directly added to the wells that contained IgM-sensitized ORBC and spleen cells. Final concentrations are shown.
‡ ORBC and spleen cells were prepared in BBS that contained 20 mM Mg++. § Data were obtained from three samples for each point. The control contained 11 ± 1% IgM-rosette-forming cells. The percentage of control was calculated as: (percentage of rosette-forming cells in sample with chelating agent/percentage of rosette-forming cells in control) × 100.

Table III

Antibody-sensitized ORBC Binding by Macrophages

| Group | BBS that contained | ORBC sensitized with | IgG | IgM | Unsensitized |
|-------|--------------------|----------------------|-----|-----|--------------|
|       |                    |                      | %   |     |              |
| 1     | No divalent cations|                      | 84* | 5   | 4            |
| 2     | 2 mM Ca**‡         |                      | 88  | 99  | 5            |
| 3     | 5 mM EDTA§         |                      | 85  | 6   | 2            |
| 4     | Reconstituted to 2 mM Ca**§ |        | 93  | 95  | 3            |

* Numbers shown are the percentage of macrophages binding three or more erythrocytes. The results shown are representative of a series of three experiments. Similar results have been obtained with resident (nonelicited) peritoneal macrophage, except that the number of cells expressing easily detectable IgG or IgM complex receptors was lower.
‡ For inhibition studies, both sensitized ORBC and macrophages were washed with the chelating agent. EGTA also inhibited IgM complex binding by macrophages.
§ Treated with EDTA as in group 3, washed with BBS, and 2 mM Ca++ added.

Macrophage binding of IgM complexes in the presence of 2 mM Ca++ was significantly reduced after washing macrophages and IgM-sensitized ORBC with 5 mM EDTA; the rosetting activity could be reconstituted by the addition of Ca++. Similar results were obtained with EGTA (data not shown). As reported by others (3, 7), neither lymphocyte nor macrophage IgG complex binding was dependent upon divalent cations. 51 ± 7% of spleen cells bound to IgG-sensitized erythrocytes in divalent cation-free BBS. This was essentially unaffected by the addition of either chelating agents or divalent cations. Similarly, 88 ± 4% of macrophages bound IgG-sensitized ORBC, irrespective of divalent cation concentration (Table III).

Discussion

We have thus demonstrated a differential divalent cation requirement for macrophage and lymphocyte Fc receptor binding that is dependent upon the type of lymphoid cells involved and the class of antibody used. Mouse lymphocytes require Mg++ but not Ca++ for optimal IgM complex binding. In contrast, macrophage rosette formation with IgM-sensitized erythrocytes is supported by Ca++ but not
Mg++. The experiments with chelating agents support these findings. Ca++ is bound equally well by either chelating agent (24). Correspondingly, inhibition of Ca++-dependent IgM complex binding to macrophages was affected equally by EDTA or EGTA. Mg++, on the other hand, has a higher affinity for EDTA than EGTA (24). Thus, Mg++-dependent IgM complex binding to lymphocytes was inhibited to a lesser degree by EGTA than EDTA. Indeed, 5 mM EGTA in the presence of 20 mM Mg++ enhanced lymphocyte IgM complex binding, probably by removing cell-bound Ca++ and allowing further Mg++ attachments to take place. IgG complex binding to both lymphocytes and macrophages, on the other hand, is divalent cation independent.

Previous results from our laboratories have indicated that the lymphocytes that bind IgM complexes through Fc receptors include both T and B cells (11). It is likely that IgM Fc receptor binding to lymphocytes and macrophages has functional significance. It has been shown, for example, that lymphocytes can function as cytotoxic effector cells against IgM-sensitized target cells (14-21). IgM antibody may also participate in thymus-dependent helper activity by IgM Fc receptor interaction (22, 23). Macrophages and T cells could be involved in such cellular collaboration by binding to IgM complexes. The molar concentration of Mg++ required for lymphocyte IgM complex binding in vitro would seem inconsistent with physiological concentrations. However, increased concentrations of Mg++ might be available at the membrane during inflammatory reactions. Mg++ and Ca++ have been implicated in distinct steps during cellular immune reactions (25-27).

The enhanced binding of IgM complexes affected by divalent cations could be a result of stereochemical modifications in the Fc region of IgM or its corresponding receptor site. Alternatively, the divalent cations could participate in the ligand interaction by forming Ca++ or Mg++ bridges between the receptor and the IgM Fc. The present data do not allow a critical distinction between these possibilities. X-ray crystallography studies that compare Ca++ and Mg++ complexes with hydrated organic molecules have shown some fundamental differences in the binding properties of these two divalent cations (24, 28). Ca++ displays a greater number of ligands (higher coordination numbers) than Mg++, and the cation ligand distances of Ca++ are greater than those of Mg++. Both Ca++ and Mg++ coordinate to oxygen atoms, whereas Mg++ also coordinates to nitrogen atoms. It is conceivable that these differences in the binding properties of Ca++ and Mg++ could be responsible for differences observed in binding of IgM to its Fc receptor on macrophages vs. lymphocytes.

Summary

We have found that Mg++ supports IgM complex binding to mouse lymphocytes but not to macrophages. In contrast, Ca++ supports IgM complex binding to macrophages but not lymphocytes. IgG complex binding to both lymphocytes and macrophages is divalent cation independent. These findings allow one to distinguish IgG from IgM binding and macrophage from lymphocyte IgM complex binding by their differential divalent cation requirements.

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