Binding and Uptake of Agalactosyl IgG by Mannose Receptor on Macrophages and Dendritic Cells

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Binding and Uptake of Agalactosyl IgG by Mannose Receptor on Macrophages and Dendritic Cells

Xin Dong,* Walter J. Storkus, ‡‡ and Russell D. Salter*‡‡

Increased levels of agalactosyl IgG (G0 IgG) are found in several autoimmune diseases, including rheumatoid arthritis, in which they are correlated with severity of the disease. To investigate whether structural alteration of IgG may lead to aberrant processing and presentation of IgG peptides as autoantigens, we have studied uptake of G0 IgG by human dendritic cells and macrophages cultured from PBMC. We found that enzymatic removal of terminal galactose residues, which exposes N-acetylglucosamine residues, increases uptake of soluble IgG mediated by mannose receptor on macrophages and dendritic cells. Efficient uptake appears to require recycling of the receptor, can be blocked by saccharides or Abs reactive with mannose receptor, and is dependent upon the state of maturation of the dendritic cells. No differences between IgG isotypes in ability to be internalized by APC were identified, suggesting that uptake would not be limited to a particular subset of Abs. These results suggest a novel pathway by which Abs or Ag-Ab complexes can be taken into dendritic cells and macrophages, and potentially generate epitopes recognized by T cells. These findings may have particular relevance for autoimmune disorders characterized by high levels of G0 IgG. * The Journal of Immunology, 1999, 163: 5427–5434.

Macrophage mannose receptor (or mannose receptor) is a C-type lectin, containing multiple carbohydrate-recognition domains, and is expressed primarily on macrophages and dendritic cells, as well as some endothelial cells (1, 2). The receptor binds glycoligands containing exposed fucose, mannose, and N-acetylglucosamine residues, including a variety of compounds containing complex carbohydrate structures (3, 4). Due to the ability of the receptor to recycle, ligand uptake by cells is essentially continuous, allowing accumulation of large quantities of ligands intracellularly (1, 5).

Ags bound by mannose receptor on dendritic cells are internalized and transported to a subcellular compartment in which Ag processing occurs (5–7). Peptide epitopes that are generated can bind to MHC proteins and subsequently be presented to Ag-specific T lymphocytes. It has been shown that addition of mannose residues to protein Ags such as BSA results in 1,000–10,000-fold increase in efficiency of presentation to Ag-specific CD4+ T cells, presumably due to much higher efficiency of internalization of the glycosylated Ag by mannose receptor (7, 8). The receptor is also responsible for uptake of the mycobacterial product lipoarabinomannan into dendritic cells, which binds to CD1b molecules and can be presented to CD4+, CD8+ T cells (6). CD1b has been shown to bind several derivatives of mycohexamic acid, and exhibits structural homology to MHC class I molecules, which present peptide Ags to CD8+ T cells (9). Both CD1b and class II MHC proteins bind Ags in a specialized vesicular compartment within APC called the MHC class II compartment, which has also been shown to contain HLA-DM, lysosomal hydrolases, and in addition mannose receptor (6, 10). These data suggest that mannose receptor can deliver a variety of Ags to the MHC class II compartment for processing and presentation by class II MHC or CD1b molecules. It is therefore important to understand the structural basis for interaction of Ags with mannose receptor, and potentially to identify novel ligands for the receptor.

In the present study, we demonstrate that the agalactosyl form of IgG (G0 IgG)3 can be internalized by mannose receptor on macrophages and dendritic cells. IgG contains a biantennary oligosaccharide attached at asparagine 297 in the Fc region. Each arm of the glycan normally terminates in galactose and sialic acid residues, and normally glycosylated IgG has been called G2 IgG (11). G0 IgG lacks these terminal residues, thereby exposing N-acetylglucosamine residues (11, 12). This results in increased solvent accessibility and conformational flexibility of the G0 glycan, allowing binding to plant lectins specific for N-acetylglucosamine, and also to serum mannose-binding protein, which plays a role in triggering complement activation by microbes (13). This latter observation led to the hypothesis that G0 IgG could contribute to the pathogenesis of rheumatoid arthritis, and additional autoimmune disorders that are characterized by the presence of high circulating levels of G0 IgG, by contributing to inflammation via complement activation (13, 14). Mannose receptor has ligand-binding properties that are similar to mannose-binding protein, and we hypothesized that uptake of G0 IgG by potent APC such as macrophages and dendritic cells would result in the accumulation of IgG molecules in Ag-processing compartments within these cells. This could potentially result in the generation of abnormally large quantities of IgG-derived peptides that bind to class II molecules. Particularly in dendritic cells, which are highly potent APC, this could produce a very strong stimulus for activation of Ig-specific T cell clones, which might otherwise remain tolerized. Such T cells could potentially provide help to B cells specific for self IgG, resulting in production of anti-IgG Abs termed rheumatoid factors. Although

3 Abbreviations used in this paper: G0 IgG, agalactosyl IgG; BS-II, Bandeiraea simplicifolia lectin; G2 IgG, galactosylated IgG; mBSA, mannosylated BSA; RCA, Ricinus communis agglutinin.
rheumatoid factors are often IgM, other isotypes have been identified, and particularly for production of the moderate to high affinity rheumatoid factors showing evidence of affinity maturation, T cell help would likely be required (15–18).

We examined whether G0 IgG could interact with mannose receptor by measuring internalization of fluorescently labeled IgG by dendritic cells and macrophages at 37°C. Uptake of G0 IgG, but not G2 IgG, was highly efficient and could be blocked either by specific sugars or Abs reactive with mannose receptor. These data demonstrate that interaction of G0 IgG with both of these cell types can be mediated by mannose receptor.

Materials and Methods

Biochemicals

FITC-celite was obtained from Sigma (St. Louis, MO). Conjugation of different proteins with FITC was done according to protocol supplied by the manufacturer. FITC-conjugated mannosylated BSA (mBSA), mannan derived from *Saccharomyces cerevisiae*, and the monosaccharides D-galactose, D-glucose, L-fucose, D-mannose, and N-acetylglucosamine were purchased from Sigma. IgG from normal human serum or derived from human plasmacytomas were purchased from either Sigma or Calbiochem (San Diego, CA).

Cell cultures

The B lymphoblastoid cell line WS was generated from a healthy donor by transformation with EBV using standard methods (19). Dendritic cells and macrophages were prepared from human adherent PBMC, as previously described (20). Briefly, PBMC were isolated from leukopheresed blood from healthy donors using density centrifugation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradients. After washing in HBSS (Life Technologies, Gaithersburg, MD), cells were resuspended in AIM-V serum-free medium (Life Technologies) at approximately 10^6/ml and incubated in T75 cell culture flasks for 1–2 h at 37°C. The plastic-adherent cells were further cultured (37°C, 5% CO2) in AIM-V medium supplemented with 1000 U/ml IL-4 and 1000 U/ml GM-CSF for dendritic cells (both cytokines were provided by Schering-Plough, Kenilworth, NJ), or 1000 U/ml GM-CSF alone (for macrophages). In most experiments, except where noted, cells were harvested for use in assays after 4 to 5 days in culture.

Antibodies

Hybridomas w6/32 (anti-HLA-AB,C), IV.3 (anti-CD32), 32.2 (anti-CD64), and 3C10 (anti-CD14) were obtained from American Type Culture Collection (Manassas, VA). FITC-conjugated mAbs reactive with CD1a, CD12, CD32, CD40, CD44, CD64, CD83, CD86, and isotype-matched controls (IgG1 and IgG2a) were obtained from PharMingen (San Diego, CA). FITC-conjugated anti-HLA-DR, CD16, CD20 mAbs were purchased from Becton Dickinson (Mountain View, CA). FITC-conjugated anti-CD80 mAb was obtained from Ancell (Bayport, MN). mAbs reactive with human mannose receptor were obtained from the following sources: clone 19 (21) from Dr. Phil Stahl (St. Louis, MO) or purchased from PharMingen; Ab 15-2-2 (22) from Dr. D. C. Rijken (Leiden, Netherlands); and 2.1D10 from Dr. Sun-sang J. Sung (Charlottesville, VA). mAb MR15 reactive with human macrophage marker CD68 was obtained as part of a typing panel for the Fifth International Workshop on Human Leukocyte Differentiation Antigens.

Generation of G0 IgG by digestion of normal human IgG

Normal human IgG was digested for 20 h at 37°C with neuraminidase (2 U/ml; Sigma) and β-galactosidase (40 U/ml; Sigma) in sodium acetate buffer (50 mM, pH 5.5) containing 10 mM MucCl2, and 0.02% NaN3 (23). The digested IgG was purified through HiTrap protein G column (Pharmacia) following the manufacturer’s protocol to remove glycosidases. *Ricinus communis* agglutinin I (RCA-I) obtained from Sigma and *Bandeiraea simplicifolia* lectin (BS-II) from E-Y Lab (San Mateo, CA) were used in agglutinin I (RCA-I) obtained from Sigma and BS-II (white bars), selective for galactose and N-acetylgalcosamine, respectively. The results shown represent the mean ± SD of triplicate wells from a representative experiment.

Flow cytometry

Cells (2 × 10^5) were incubated with mAbs specific for cell surface markers for 45 min at 4°C. After two washes in PBS containing 1% BSA, FITC-conjugated goat anti-mouse IgG Ab (Sigma) was added for 30 min. Cells were then washed three times in PBS, then fixed with 4% paraformaldehyde in PBS. Samples were then analyzed by FACScan (Becton Dickinson), and mean fluorescence channels were recorded.

Quantitative analysis of ligand uptake by cells

Cells were harvested by gentle pipetting from flasks, and resuspended in RPMI 1640 medium (without serum) at 5 × 10^6/ml. Fluorescein-labeled ligands were diluted in RPMI medium, then added to 2.5 × 10^6 resuspended cells, followed by incubation at either 37°C or 4°C. For studies using inhibitors, mAbs or saccharides were added at the onset of incubation. Cells were washed three times in PBS, then fixed with 4% paraformaldehyde in PBS before examination by flow cytometry or fluorescence microscopy. Flow-cytometric data were collected as described above.

Results

Generation of G0 IgG by glycosidases

To generate G0 IgG, we used the exoglycosidases β-galactosidase and neuraminidase to remove the terminal galactose and sialic acid residues from normal G2 IgG, resulting in exposure of N-acetylgalcosamine residues. To test the efficiency of digestion, control and digested IgG preparations were analyzed by ELISA for binding to the plant lectins RCA-I and BS-II, which are selective for galactose and N-acetylgalcosamine, respectively. As shown in a representative experiment, digested IgG showed decreased RCA-I binding and increased BS-II binding compared with native IgG (Fig. 1). We estimated that greater than 75% of galactose residues on IgG were removed typically by digestion.

Generation and phenotypic analysis of macrophages and dendritic cells

Dendritic cells and macrophages derived from PBMC used in these studies were analyzed for expression of a number of cell surface markers, as shown in Table I. Dendritic cells cultured for 4 to 5 days expressed CD32, CD40, CD44, CD86, MHC class I and class II, mannose receptor, and low levels of CD80 and CD1a, and were negative for CD14, CD20, CD64, CD68, and CD83. This surface marker profile is consistent with a relatively immature phenotype shown previously to be characteristic of dendritic cells that

![FIGURE 1. Removal of the terminal sialic acid and galactose residues from G2 IgG to generate G0 IgG. IgG was digested with neuraminidase and β-galactosidase, and then assayed by ELISA, as described in Materials and Methods, using the lectins RCA (black bars) and BS-II (white bars), selective for galactose and N-acetylgalcosamine, respectively. The results shown represent the mean ± SD of triplicate wells from a representative experiment.](http://www.jimmunol.org/Downloadedfrom/...)
efficiently take up and process exogenous Ags. Macrophages cultured from the same preparation of PBMC as described in Materials and Methods expressed CD14, CD68, CD32, mannose receptor, and weakly CD64. This distinct pattern of surface marker expression is consistent with previous analyses of cultured macrophages. Cell cultures routinely contained 85–95% dendritic cells or macrophages based on phenotyping and morphology, with the remainder consisting mainly of T cells.

**G0 IgG is internalized by human dendritic cells and macrophages**

Dendritic cells and macrophages were cultured for 5 days and then analyzed for expression of cell surface markers or uptake of ligands, together with a B lymphoblastoid cell line derived from the same donor. Fig. 2 shows that all three lines express CD32, but that only macrophages and dendritic cells express mannose receptor. Cells were incubated with fluoresceinated ligands at either 37°C or 4°C, as shown in Fig. 3. Incubation at 37°C, but not at 4°C, resulted in significant cell fluorescence, suggesting that the ligand is internalized at 37°C. As previously reported, mannosylated BSA (mBSA) is taken up much more efficiently than unmodified BSA into both dendritic cells (Fig. 3a) and macrophages (Fig. 3b) after incubation for 1 h at 37°C. In contrast, exposure of B cell lines to fluoresceinated mBSA, which do not express mannose receptor, did not result in significant labeling of cells (Fig. 3c).

Both dendritic cells and macrophages were strongly labeled following incubation with fluoresceinated G0 IgG, but not with the same concentration of fluoresceinated G2 IgG. As with mBSA, labeling occurred only following incubation at 37°C, and not at 4°C. Fluoresceinated G0 IgG was present primarily in vesicles in dendritic cells within 15 min after exposure to the ligand, and could not be detected at the cell surface, confirming that G0 IgG is internalized by cells (unpublished data). The WS B cell line was also exposed to fluoresceinated G0 IgG, but was not significantly labeled, consistent with the observations using mBSA. These results have been confirmed with cells from multiple donors (Table II), and demonstrate that G0 IgG is taken up selectively by dendritic cells and macrophages. Since the uptake of both G0 IgG and mBSA depended upon their glycosylation status, these results supported the hypothesis that a receptor with lectin-like properties such as mannose receptor was required.

**Time and dose dependence of G0 IgG uptake**

When dendritic cells were incubated with 40 μg/ml of fluoresceinated G0 IgG, internalization increased over the incubation period without reaching a plateau (Fig. 4). This demonstrated that uptake was highly efficient, and would be consistent with the previously reported ability of mannose receptor to bind and internalize ligands, then recycle to the plasma membrane (27, 28). Dendritic cells incubated for 1 h with increasing concentrations of ligands were analyzed (Fig. 5), and showed that exposure to G0 IgG resulted in the cells becoming increasingly fluorescent. Although this suggests that G0 IgG uptake is nonsaturable, it may simply reflect limitations in the concentration of ligand that are achievable. Uptake of G0 IgG by dendritic cells was inhibited with 100 μg/ml mannan, which has been used previously to block uptake of other ligands by mannose receptor (5). In contrast, mannan did not significantly inhibit uptake of aggregated G2 IgG (Table III), which is internalized by Fc receptors on dendritic cells and macrophages.

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**Table I. Phenotype of dendritic cells and macrophages following 4 days in culture**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Expt. 1</th>
<th>Expt. 2</th>
<th>Expt. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Macrophage</td>
<td>DC</td>
<td>Macrophage</td>
</tr>
<tr>
<td>Neg (IgG1)</td>
<td>2 (0.1)</td>
<td>3 (0.2)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>Neg (IgG2)</td>
<td>1 (0.1)</td>
<td>2 (0.5)</td>
<td>6 (1)</td>
</tr>
<tr>
<td>CD1a</td>
<td>2 (0.1)</td>
<td>31 (30)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>CD14</td>
<td>70 (79)</td>
<td>4 (0.3)</td>
<td>112 (78)</td>
</tr>
<tr>
<td>CD20</td>
<td>3 (1)</td>
<td>2 (0.2)</td>
<td>4 (1)</td>
</tr>
<tr>
<td>CD32</td>
<td>52 (92)</td>
<td>84 (95)</td>
<td>48 (87)</td>
</tr>
<tr>
<td>CD40</td>
<td>37 (92)</td>
<td>93 (89)</td>
<td>52 (89)</td>
</tr>
<tr>
<td>CD44</td>
<td>68 (98)</td>
<td>89 (99)</td>
<td>39 (90)</td>
</tr>
<tr>
<td>CD64</td>
<td>8 (1)</td>
<td>3 (1)</td>
<td>6 (1)</td>
</tr>
<tr>
<td>CD68</td>
<td>45 (77)</td>
<td>2 (1)</td>
<td>38 (81)</td>
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<td>CD80</td>
<td>14 (32)</td>
<td>60 (91)</td>
<td>23 (41)</td>
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<tr>
<td>CD83</td>
<td>1 (1)</td>
<td>2 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>CD86</td>
<td>80 (84)</td>
<td>97 (99)</td>
<td>72 (90)</td>
</tr>
<tr>
<td>MHC I</td>
<td>482 (98)</td>
<td>511 (99)</td>
<td>295 (91)</td>
</tr>
<tr>
<td>MHC II</td>
<td>258 (91)</td>
<td>439 (97)</td>
<td>214 (89)</td>
</tr>
<tr>
<td>MR</td>
<td>36 (78)</td>
<td>28 (35)</td>
<td>42 (76)</td>
</tr>
</tbody>
</table>

*a Values are expressed as mean fluorescence intensity (percentage of positive cells).
*b DC, dendritic cell.
This confirms that mannan is able to selectively block uptake of carbohydrate ligands, as previously demonstrated (5). Similar results were obtained using macrophage preparations (unpublished data).

Uptake of G0 IgG can be inhibited by some monosaccharides and Abs against mannose receptor

To further characterize the interaction of G0 IgG with dendritic cells, uptake of either G0 IgG or mBSA was measured in the presence of 100 mM monosaccharides (Fig. 6). Uptake of both ligands was blocked by mannose and fucose, and somewhat less well by N-acetylglucosamine. Galactose was unable to inhibit uptake, either for G0 IgG (Fig. 6a) or for mBSA (unpublished data). The pattern of inhibition is consistent with uptake being mediated by mannose receptor, based on previously published studies. To provide further evidence for a role for the receptor, we measured uptake of labeled ligands by dendritic cells in the presence of Abs reactive with mannose receptor (Fig. 6b). Ab 19 used at the highest concentration tested (10 μg/ml) inhibited uptake of G0 IgG and mBSA by 69% and 55%, respectively, while Abs 15-2-2 and 2.1D10 blocked only slightly at this concentration. At higher concentrations, clone 19 blocked internalization of both ligands by 70 – 80% (unpublished data). All three Abs bound to dendritic cells comparably, as determined by flow cytometry (unpublished data). These results strongly support the conclusion that uptake of G0 IgG and mBSA are both mediated primarily by mannose receptor. Although the lack of complete inhibition indicates that a secondary mechanism for uptake of these ligands may exist, we believe it equally possible that the highly efficient nature of mannose receptor uptake and recycling would be difficult to block completely with Abs, perhaps due to internalization of the specific Ab itself.

G0 IgG uptake is not dependent on Ig subclass

To determine whether the subclass of IgG influenced its ability to interact with mannose receptor following removal of galactose residues, we subjected nine different preparations of IgG isolated from myelomas grown in culture to enzymatic digestion to remove galactose residues. Table IV shows the g-chain subclass and light chain isotype of each preparation, and compares the ratio of BS-II to RCA lectin binding following digestion to uptake of fluoresceinated ligand by dendritic cells. There were significant differences in the ability of individual preparations to be digested, with IgG in some samples appearing to be resistant to digestion, as

<table>
<thead>
<tr>
<th>DC Preparation</th>
<th>G2 IgG (40 μg/ml)</th>
<th>G0 IgG (40 μg/ml)</th>
<th>mBSA (5 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.3*</td>
<td>39.8</td>
<td>89.8</td>
</tr>
<tr>
<td>2</td>
<td>4.9</td>
<td>116.1</td>
<td>289.9b</td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
<td>59.9</td>
<td>106.4</td>
</tr>
<tr>
<td>4</td>
<td>5.1</td>
<td>35.4</td>
<td>82.7</td>
</tr>
</tbody>
</table>

* Mean fluorescence intensity.

b 10 μg/ml.

FIGURE 4. Uptake of G0 IgG by dendritic cells (DC) increases with time of incubation. Dendritic cells were incubated for the times incubated with 40 μg/ml FITC-conjugated G0 IgG at 37°C, and removed for immediate washing and fixation. Flow cytometry was used to analyze uptake as in Fig. 3. Data shown here are representative of more than three experiments.
indicated by a low ratio of BS-II/RCA binding, even after redigestion. However, in these samples, binding of both lectins was quite low relative to total protein, suggesting that the preparations might contain significant amounts of IgG with missing or aberrant glycan structures that would not interact with lectins, including mannose receptor (unpublished data). A clear correlation was seen between efficient digestion to obtain G0 IgG and the level of uptake of each IgG preparation. Furthermore, IgG1, IgG2, and IgG3 preparations bearing either κ- or λ-light chains were each able to be efficiently converted to the G0 form and subsequently internalized by dendritic cells. We were unable to obtain additional IgG4 preparations, and thus cannot further assess this subclass. These results suggest that Ig subclass and light chain type do not influence uptake by mannose receptor.

Mannose receptor-mediated endocytosis is correlated with the maturation stage of dendritic cells

As dendritic cells mature, either in vivo or in vitro, they undergo phenotypic changes in cell surface protein expression and also in their ability to internalize ligands. In the previous experiments, we examined dendritic cells grown in vitro for 4 to 5 days that have an immature phenotype, characterized by the ability to take up Ags very efficiently via macropinocytosis, receptor-mediated endocytosis, or phagocytosis (5, 29, 30). As previously reported, immature dendritic cells treated with LPS, which induces a more mature phenotype, undergo a rapid down-modulation in their ability to internalize mBSA (5). A similar process of maturation occurs spontaneously with increasing time in culture in the absence of LPS or other stimulatory substances, although the phenotypic changes appear more gradually. To examine whether mannose receptor expression and function change over time, we isolated dendritic cells at different times after initiation of culture, and measured the expression of several surface markers, including mannose receptor, using specific Abs and also the ability of cells to internalize labeled G0 IgG or mBSA (Fig. 7). Between days 2 and 6, mannose receptor expression was high, and both ligands were internalized. At days 8 and 10, however, ligand uptake had stopped, although mannose receptor expression was still quite high, as detected by specific two Abs. This experiment demonstrates that mannose receptor is active in immature dendritic cells, at different times after initiation of culture, and measured the expression of several surface markers, including mannose receptor, using specific Abs and also the ability of cells to internalize labeled G0 IgG or mBSA (Fig. 7). Between days 2 and 6, mannose receptor expression was high, and both ligands were internalized. At days 8 and 10, however, ligand uptake had stopped, although mannose receptor expression was still quite high, as detected by specific two Abs. This experiment demonstrates that mannose receptor is active in immature dendritic cells,

Table III. Inhibition of G0 IgG uptake by dendritic cells in the presence of mannan

<table>
<thead>
<tr>
<th>IgG Concentration (μg/ml)</th>
<th>Aggregated G2a</th>
<th>% Inhibition</th>
<th>Aggregated G0</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4a</td>
<td>NA</td>
<td>4</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>&lt;0</td>
<td>13</td>
<td>44</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>&lt;0</td>
<td>23</td>
<td>65</td>
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<td>106</td>
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<td>78</td>
</tr>
<tr>
<td>60</td>
<td>200</td>
<td>35</td>
<td>97</td>
<td>78</td>
</tr>
</tbody>
</table>

*a IgG was aggregated by cross-linking with Cy3 bisfunctional dye (Amersham, Pittsburgh, PA), which was also used as the fluorochrome for detection of ligand uptake. SDS-PAGE was used to confirm cross-linking of the protein. Dendritic cells were incubated with the indicated ligands in the presence of 100 μg/ml mannan, as described in Fig. 5.

*b Mean fluorescence intensity.
as measured by ligand uptake, but its function decreases drastically following maturation of cells to a phenotype that expresses higher levels of CD86 and cell surface class II MHC (30–32). In addition, it is clear that the presence of mannose receptor on the plasma membrane is not sufficient for receptor function, since dendritic cells express the receptor following 8 days in culture (Fig. 7), but do not take up ligand.

### Table IV. Uptake of different IgG isotypes by dendritic cells

<table>
<thead>
<tr>
<th>Myeloma Subclass</th>
<th>G0 IgG</th>
<th>G2 IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ratio of BS-II/RCA Binding</td>
<td>Uptakea</td>
</tr>
<tr>
<td></td>
<td>Ratio of BS-II/RCA Binding</td>
<td>Uptakea</td>
</tr>
<tr>
<td>1 IgG1, κ</td>
<td>0.3</td>
<td>7.5</td>
</tr>
<tr>
<td>2 IgG1, λ</td>
<td>45.9</td>
<td>33.9</td>
</tr>
<tr>
<td>3 IgG1</td>
<td>≥100μ</td>
<td>163.8</td>
</tr>
<tr>
<td>4 IgG2, κ</td>
<td>≥100μ</td>
<td>101.2</td>
</tr>
<tr>
<td>5 IgG2, λ</td>
<td>5.5</td>
<td>141.9</td>
</tr>
<tr>
<td>6 IgG3, κ</td>
<td>0</td>
<td>5.6</td>
</tr>
<tr>
<td>7 IgG3, λ</td>
<td>≥100μ</td>
<td>94.9</td>
</tr>
<tr>
<td>8 IgG3</td>
<td>≥100μ</td>
<td>99.1</td>
</tr>
<tr>
<td>9 IgG4, κ</td>
<td>0</td>
<td>11.0</td>
</tr>
</tbody>
</table>

a Mean fluorescence intensity.

b Lectin binding ratio ≥100 indicates no detectable RCA binding.

c Lectin binding ratio ≤0.01 indicates no detectable BS-II binding.

### Discussion

We have identified a novel ligand for mannose receptor, namely G0 IgG, which is present in abundance in the serum of some patients with rheumatoid arthritis and other autoimmune diseases, particularly when the disease is progressive (12, 33). In addition, mouse strains such as lpr, which are genetically predisposed to autoimmunity, have an abnormally high proportion of G0 IgG (34). These observations are consistent with a role for G0 IgG in disease pathogenesis, a hypothesis that has been further supported by experiments in which arthritis-type symptoms can be transferred by infusion of G0 IgG into nondiseased murine recipients (35). In the latter model, DBA/2 mice are first immunized with collagen to induce arthritis. Abs reactive with collagen are purified from the sera of these mice, and digested to generate G0 IgG. Abs are mixed with collagen, and infused into nondiseased animals previously immunized with collagen. Arthritis symptoms appear when the G0 form, but not the G2 form, of the anti-collagen Ab is used. Several possible explanations for transfer of disease symptoms with G0 IgG can be suggested, including altered effector functions, altered catabolism, or altered antigenicity.

Our results are potentially relevant to the last possibility, since dendritic cells, which are among the most potent APC in the body, and macrophages can take up large amounts of G0 IgG via mannose receptor. This may lead to processing and presentation of IgG-derived peptides in class II MHC, which could stimulate

![FIGURE 7](http://www.jimmunol.org/)
CD4+ T cell responses. Although T cells are selected to be tolerant of self proteins, presumably including IgG, uptake of much larger amounts than normal by APC could potentially break tolerance by stimulating T cells cross-reacting with IgG peptides. It is also possible that uptake of IgG by mannose receptor results in its shunting to intracellular compartments, in which processing is altered such that normally unexposed or cryptic epitopes are revealed. Precedents for such altered processing have been reported recently, in which generation of particular epitopes depends upon the receptor used for Ag uptake (36).

In any case, to effect an immune response, IgG-derived peptides would have to bind to class II MHC proteins and be transported to the surface of the APC, before being able to stimulate T cell responses. To address the question directly then, it will be necessary to determine whether peptides from IgG can be generated and bind to class II MHC proteins. This has been determined for the human class II molecule HLA-DR4 expressed on B cell lines, which binds to class II MHC proteins. This has been determined for the human class II molecule HLA-DR4 expressed on B cell lines, which binds peptides 145–159 and 188–203 from endogenously synthesized κ-light chains (37, 38). These peptides bind to HLA-DR4 subtypes that are associated with rheumatoid arthritis, suggesting a particular relevance to the disease. However, it is not known if other APC, such as dendritic cells and macrophages, process IgG into the same or different peptides, or if IgG taken up exogenously by these cells would lead to differential processing. Ultimately, it will be necessary to determine whether APC generate IgG peptides that can bind to class II MHC proteins, and then stimulate T cell immune responses in individuals with autoimmune disorders.

The results presented in this study also have important implications for understanding how potential Ags other than IgG may be taken into macrophages and dendritic cells. It is likely that a variety of self proteins have glycanside chains that can interact with mannose receptor, including but not limited to those with fucose, mannose, and N-acetylgalosamine. Most of these ligands, even if taken up by APC in large quantities, would not be expected to provoke an immune response since T cells appropriately selected in the thymus should be tolerant of peptides derived from self proteins. However, if the amount of the protein taken into a potent APC changed drastically, as seen in tissue culture for G0 IgG uptake by macrophages and dendritic cells, it might be possible to break self-tolerance. It is not clear what alters the glycosylation state of IgG in the disease, or if other less abundant proteins have altered glycosylation in rheumatoid arthritis or other disease states. A further possibility is that immune complexes containing G0 IgG might allow internalization of Ags bound to specific IgG via the IgG-combining site. This could permit increased uptake of even nonglycosylated proteins indirectly via specific Ab and mannose receptor. In therapeutic situations in which it would be desirable to enhance Ag uptake for use in vaccines, it may be possible to exploit this effect by generating the G0 form of Ab against a desired Ag for targeting into APC.

Abs reactive with mannose receptor that we tested have differential ability to interfere with uptake of ligands, with some blocking quite efficiently, while others promoted uptake to a slight extent (Fig. 6). This suggests the interesting possibility that the latter set might be used to stimulate uptake of Ags by mannose receptor-positive cells, either by addition of the Ag, or by covalent coupling of Ag and receptor-specific Ab. In mice, uptake of Ags via the receptor DEC-205, which is structurally related to mannose receptor, was facilitated by conjugation of a peptide Ag to an Ab against DEC-205 (39). Nonblocking Abs against mannose receptor in humans may be useful for promoting Ag uptake in a similar way.

In addition to identifying a novel ligand for mannose receptor, this study demonstrates that dendritic cells alter their ability to internalize ligands via mannose receptor during maturation. This is consistent with previous reports suggesting that Ag uptake is stopped once dendritic cells mature from a processing to a presenting phenotype, either by exposure to inflammatory stimuli such as LPS or TNF-α, or with increasing time in culture (5). In our study, dendritic cells cultured for 8 or 10 days were shown to express mannose receptor, but were unable to internalize ligands via the receptor. This suggests that cells at this stage of maturation are poised between the processing and presenting phenotypes. In addition, this result implies that the presence of mannose receptor on the plasma membrane is not sufficient for its function. It may be that internalization via mannose receptor requires additional molecules that are not present in our dendritic cell cultures at day 8, or that broader changes are involved, such as rearrangement of cytoskeletal elements during cell maturation.

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References


