Suppression of Polyclonal Immunoglobulin Production by M-proteins Shows Isotype Specificity

Liang Wang and David C. Young
Department of Pathology, University of Texas Houston Health Science Center, Houston, Texas

Abstract. Monoclonal gammopathies are B cell neoplasms that are characterized by the presence of monoclonal immunoglobulins (M-proteins) in the serum. By an unknown mechanism, the normal polyclonal immunoglobulin levels are frequently reduced in sera of these patients. To assess the role of M-protein isotype in this effect, we used serum protein electrophoresis to quantitate monoclonal and polyclonal immunoglobulins in patients and we used serum immunofixation electrophoresis to determine their M-protein isotype. When divided into populations of 30 patients with IgG M-proteins (mean 2.5 g/dl) and 19 patients with IgM or IgA M-proteins (mean 2.6 g/dl), the mean polyclonal immunoglobulin level was significantly lower in the IgG M-protein population (0.4 g/dl) than the IgM/IgA population (0.8 g/dl). Patients with IgG M-proteins also had significantly lower polyclonal immunoglobulin levels when compared separately with the patients with either IgA or IgM paraproteins. Since the polyclonal immunoglobulin fraction is comprised mostly of IgG, these results give the first direct indication that IgG M-proteins have a greater suppressive effect on polyclonal IgG levels than do M-proteins of other isotypes. These findings suggest that an isotype-specific feedback mechanism could be involved in the normal regulation of serum IgG levels. (received 2 May 2001; accepted 7 May 2001)

Keywords: M-protein, paraprotein isotype, polyclonal immunoglobulins, immune regulation

Introduction

Monoclonal gammopathy is caused by a B cell lineage neoplastic cell line that produces immunoglobulins or components of immunoglobulins (M-proteins, para-proteins). M-proteins may be of any immunoglobulin isotype, but most commonly are IgG, IgM, or IgA. They are infrequently composed only of heavy or light chains. Monoclonal immunoglobulins may be detectable by serum protein electrophoresis as bands that are generally in the gamma or beta globulin regions, because of the over-abundance of a specific immunoglobulin idiotype. The monoclonal band is usually seen on a background of diffusely distributed polyclonal immunoglobulins of diverse idiotypes, as previously reviewed [1,2].

Polyclonal immunoglobulin levels tend to be reduced (immunoparesis) in sera of patients with monoclonal gammopathies [3,4]. In early studies, radioiodinated IgG was used to compare the rates of IgG degradation (T1/2) in patients with IgG myeloma versus patients with macroglobulinemia [5]. Lippincott et al [6] showed that 6 myeloma patients with monoclonal bands in the gamma fraction had longer IgG T1/2 values, compared to 4 patients with bands that migrated in the beta region. This suggested that IgG M-proteins caused greater suppression of polyclonal IgG than did other isotypes, but the actual isotypes were not determined and M-proteins were not quantitated. Solomon et al [7] compared the rate of loss of labeled IgG in normal controls, patients with IgG myeloma, and patients with IgM myeloma. The average degradation rate of IgG was higher in IgG myeloma patients (4.1 g/kg/da) than in macroglobulinemia patients (2.6 g/kg/da) or normal controls (3 g/kg/da). However, paraprotein levels were not matched in the 2 patient populations. The average M-protein level was higher in the IgG myeloma patients (5 g/dl) than in the macroglobulinemia patients (4 g/dl). Therefore, the higher rate of IgG degradation in IgG myeloma patients versus macroglobulinemia patients was potentially attributable to higher M-protein levels.
In more recent studies, patients with IgG M-proteins were found to produce lower anti-pneumococcal IgG antibody titers than patients with IgA or light-chain-only M-proteins [3]. In addition, levels of nonparaprotein IgG subclasses were significantly lower in patients with IgG M-proteins when compared with those with IgA or light-chain-only M-proteins [3]. These findings suggested that the suppression of polyclonal immunoglobulin production in patients with monoclonal gammopathies is isotype specific, but the levels of M-proteins were not indicated [3]. Therefore it was impossible to evaluate the effects of M-proteins on polyclonal immunoglobulins relative to the M-protein level.

In this study, we directly compared IgG with IgA and IgM M-proteins for their effects on polyclonal immunoglobulin levels. Since polyclonal immunoglobulins are comprised largely of IgG, the results address the question of whether the immunoparesis in monoclonal gammopathies is specific for the isotype of the M-protein. We found that IgG M-proteins suppressed polyclonal immunoglobulins to a greater extent than did non-IgG M-proteins (IgA and IgM).

Materials and Methods

Patients. We initially considered all of the patients in our files who had M-protein levels greater than 1 g/dl. Among the initial group of patients, those with IgG M-proteins had much higher mean M-protein levels than the patients with IgM M-proteins, making comparisons of the groups difficult. Therefore, the patients with the highest IgG M-protein levels were sequentially eliminated until the average M-protein levels of the IgG- and IgM-groups were approximately equal. Likewise, to compare the IgG M-protein group with the combined IgA and IgM M-protein groups, the patients with the highest IgG M-protein levels were sequentially eliminated until the average M-protein levels of the IgG and IgM/A groups were similar. Characteristics of the patient populations are shown in Table 1.

**Table 1. Characteristics of patients enrolled in the study.**

<table>
<thead>
<tr>
<th>Gender</th>
<th>M-protein</th>
<th>Number of patients</th>
<th>Age of patients (yr) (mean &amp; range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>female</td>
<td>IgG</td>
<td>23</td>
<td>63 (50–90)</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>4</td>
<td>60 (47–77)</td>
</tr>
<tr>
<td></td>
<td>IgA</td>
<td>3</td>
<td>70 (52–86)</td>
</tr>
<tr>
<td>male</td>
<td>IgG</td>
<td>7</td>
<td>77 (58–84)</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>6</td>
<td>72 (53–94)</td>
</tr>
<tr>
<td></td>
<td>IgA</td>
<td>6</td>
<td>68 (52–86)</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>49</td>
<td></td>
</tr>
</tbody>
</table>

**Determination of monoclonal and polyclonal immunoglobulin levels.** Serum protein electrophoresis was performed using the "Paragon" protein electrophoresis system (Beckman-Coulter Co., Fullerton, CA) according to the manufacturer's instructions. The M-protein levels and polyclonal immunoglobulin levels were determined by quantitation of the M-protein peak and the gamma fraction exclusive of the M-protein peak, based on densitometer scans of serum protein electrophoresis gels using a Beckman "Appraise" densitometer.

**Table 2. Relationships between M-protein and polyclonal immunoglobulin levels in sera from patients with IgG, IgA, or IgM monoclonal gammopathies.**

<table>
<thead>
<tr>
<th>Isotype of M-protein</th>
<th>Serum M-protein level (g/dl) (mean &amp; range)</th>
<th>Serum polyclonal Ig level (g/dl) (mean &amp; range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparison of IgG with IgA or IgM M-protein patients</td>
<td>IgG 2.5 (1.4–5.6) 0.40 (0.09–0.94)</td>
<td>IgA or IgM 2.6 (1.0–6.0) 0.80* (0.40–1.80)</td>
</tr>
<tr>
<td>Comparison of IgG with IgA M-protein patients</td>
<td>IgG 2.5 (1.4–5.6) 0.40 (0.09–0.94)</td>
<td>IgA 3.3 (1.0–6.0) 0.74* (0.40–1.79)</td>
</tr>
<tr>
<td>Comparison of IgG with IgM M-protein patients</td>
<td>IgG 2.0 (1.4–2.6) 0.37 (0.30–0.51)</td>
<td>IgM 2.0 (1.0–4.2) 0.87* (0.56–1.40)</td>
</tr>
</tbody>
</table>

* p <0.05 by t-test versus patients with IgG M-protein.
Determination of M-protein isotypes. M-protein isotypes were determined using the “Paragon” serum immunofixation system (Beckman-Coulter Co., Fullerton, CA).

Results

In this study, we tested the hypothesis that IgG M-proteins suppress production of polyclonal immunoglobulin to a greater extent than do IgA or IgM M-proteins. In order to compare serum IgG M-proteins with serum IgA or IgM M-proteins for their effects on polyclonal immunoglobulin levels, the patient populations were selected to achieve comparable mean concentrations of serum M-proteins.

Initially the levels of polyclonal immunoglobulins in patients with IgG M-proteins were compared with those of patients with non-IgG M-proteins, IgA or IgM. The mean IgG M-protein level in 30 patients was 2.5 g/dl and that in 19 patients with IgM or IgA M-proteins was 2.6 g/dl (Table 2). The mean polyclonal immunoglobulin level in patients with IgA or IgM M-proteins was 0.80 g/dl, which was significantly higher than the value of 0.40 g/dl in patients with IgG M-proteins (p = 0.0001 by t-test) (Fig. 1).

To compare polyclonal immunoglobulin levels in patients with IgG M-proteins to those with IgM M-proteins, the patients with highest IgG M-protein levels was 2.5 g/dl and that in 19 patients with IgM or IgA M-proteins was 2.6 g/dl (Table 2). The mean polyclonal immunoglobulin level in patients with IgA or IgM M-proteins was 0.80 g/dl, which was significantly higher than the value of 0.40 g/dl in patients with IgG M-proteins (p = 0.0001 by t-test) (Table 2).

Since IgG M-proteins were associated with greater suppression of polyclonal immunoglobulins than non-IgG M-proteins (IgM and IgA), it was of interest to compare the polyclonal immunoglobulin levels of IgA and IgM M-protein populations separately with those of the IgG M-protein population. The mean IgA M-protein concentration in 9 patients was 3.3 g/dl and the mean polyclonal immunoglobulin concentration was 0.74 g/dl. The mean polyclonal immunoglobulin level of this population was significantly higher than in 30 patients who had a mean IgG M-protein level of 2.5 g/dl and a mean polyclonal immunoglobulin level of 0.40 g/dl (p = 0.04 by t-test).

To compare polyclonal immunoglobulin levels in patients with IgG M-proteins to those with IgM M-proteins, the patients with highest IgG M-protein levels...
were sequentially eliminated until the mean IgG M-protein level was equivalent to that of the IgM M-protein population (ie, 1.96 g/dl). The average polyclonal immunoglobulin level for 10 IgM M-protein patients was 0.87 g/dl, which was significantly higher than the value of 0.37 g/dl obtained in 21 patients with IgG M-proteins (p = 0.0006 by t-test).

Since IgG M-proteins generally migrate more cathodally than IgM and IgA M-proteins, we wished to avoid the possibility that this effect might skew the results of polyclonal immunoglobulin levels. Therefore the IgG samples were chosen that most closely matched the IgA and IgM samples in regard to the distance of migration, measured from the β2 globulin peak to the M-protein peak. This made the distances of migration approximately equal for the 10 IgG M-proteins that were compared with 10 IgM M-proteins and the 7 IgG M-proteins that were compared with 7 IgA M-proteins (Table 3). Two IgA M-protein samples were eliminated from this analysis because there were no IgG M-proteins that were comparable in distance of migration. In this analysis, polyclonal immunoglobulin levels were still significantly lower in the IgG M-protein samples than in the IgM (p = 0.0002 by t-test) or IgA (p = 0.046) M-protein samples. Thus, the groups of patients with serum IgG M-proteins had lower polyclonal immunoglobulin levels than did groups with similar or higher serum concentrations of non-IgG M-proteins.

Discussion

The objective of this study was to determine whether the immunoparesis associated with monoclonal gammopathies is isotype specific. We addressed this question by evaluating the relative effects of M-proteins on polyclonal immunoglobulins in patients with monoclonal gammopathies. Our approach was based on the preponderance of IgG in the normal polyclonal immunoglobulin fraction. The average normal IgG level in adults is 1158 mg/dl, while that for IgM is 99 mg/dl, and that for IgA is 200 mg/dl [8]. Since about 80% of normal polyclonal immunoglobulin is IgG, a reduction in polyclonal IgG production would likely be reflected in the overall level of polyclonal immunoglobulin. Therefore, if IgG M-proteins suppress polyclonal IgG levels to a greater extent than do IgM or IgA M-proteins, one would expect greater reductions of polyclonal immunoglobulin levels with IgG than with IgM or IgA M-proteins, and this was indeed found to be the case.

These findings imply an isotype-specific mechanism for suppression of immunoglobulin production and may help to explain the observation of Fahey et al [9] that macroglobulinemia patients had fewer infectious complications than multiple myeloma patients. In another study, reduced polyclonal immunoglobulin levels in patients with monoclonal gammopathy were associated with increased incidence of serious infections [3].

Our results do not address the question of whether the suppressive mechanism is a normal feedback system for control of immunoglobulin levels, or if it is specific to monoclonal gammopathies. In our study, linear regression analysis did not show significant correlation between M-protein levels and polyclonal immunoglobulin levels (data not shown). This suggests that M-protein levels per se did not cause the reduction in polyclonal immunoglobulin levels. Chemotherapy with melphalan caused correction of suppressed immunoglobulins in about 25% of patients that responded to therapy [10], which suggests that reduction of malignant cell mass or production of M-protein may influence the production of polyclonal immunoglobulins.

A mechanism for M-protein isotype-specific suppression of polyclonal immunoglobulin is suggested by studies in which mouse IgA plasmacytomas were associated with increased numbers of T cells bearing cell surface IgA receptors [11,12]. Similar results were obtained in mice given IgG- and IgM-secreting plasmacytomas [13]. T cells bearing IgA receptors were increased by injection of IgA [14]. These results suggest that isotype-specific suppression of polyclonal immunoglobulin production could occur through T cells bearing isotype-specific immunoglobulin receptors. It seems likely that such a mechanism would also be involved in the normal feedback control of antibody production.

Our results provide the first direct evidence of isotype-specific reduction of polyclonal immunoglobulin levels in monoclonal gammopathy. This work confirms and extends the findings of Lippincott et al [6] and Solomon et al [7].

Polyclonal Ig levels in monoclonal gammopathy
Acknowledgements

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