Suppression of the humoral immune response by mycophenolate mofetil

Kenneth G. C. Smith¹,², Nicole M. Isbel¹, Michael G. Catton³, Jennie A. Leydon³, Gavin J. Becker¹ and Rowan G. Walker¹

¹Department of Nephrology, Royal Melbourne Hospital, Parkville, Victoria, Australia, ²Department of Medicine, University of Cambridge School of Clinical Medicine, Addenbrooke’s Hospital, Cambridge, UK, and ³Victorian Infectious Diseases Reference Laboratory, Fairfield, Victoria, Australia

Abstract

Background. No conventional immunosuppressive agent preferentially inhibits antibody production. Studies in experimental animals and in human cells in vitro suggested mycophenolate mofetil (MMF) might have such an effect. If this was the case in vivo it could have significant implications in terms of both MMF toxicity and the rational design of immunotherapeutic regimens.

Methods. Subjects were renal transplant recipients (25 patients treated with prednisolone, cyclosporine and azathioprine, and 13 treated with prednisolone, cyclosporine and MMF) and 20 normal controls. The three groups received influenza vaccination, and the antibody response to it was measured 4–6 weeks later using a standard haemagglutination assay.

Results. MMF profoundly suppressed the humoral immune response to influenza vaccination when added to prednisolone and cyclosporine. This effect could be seen when comparing the rise in the mean titre of antibody after vaccination. It was also reflected in the number of patients mounting responses deemed to be clinically protective by either demonstrating a 4-fold rise in titre or an increase in titre to ≥40.

Conclusions. Suppression of the humoral immune response by MMF has implications for the design of immunization protocols to protect the immunosuppressed, and raises the possibility that MMF use may be accompanied by more or different infections than complicate more conventional immunosuppressive agents.

Key words: antibodies; humoral immunity; influenza vaccination; mycophenolate mofetil; renal transplantation

Introduction

The search for immunosuppressive regimens tailored for different clinical indications has long been a major aim of therapeutic immunology. At present most immunosuppressive drugs produce broad suppression of the immune response, and in particular of T cell-mediated immunity. The most intensively studied aspect of current immunosuppressives is their effect on T cell function, and in fact many recently developed immunosuppressives act specifically on T cells (e.g. cyclosporine and FK506). The effect on the human humoral immune system of most immunosuppressive agents is incompletely understood, and none with a particular predilection for suppressing antibody production in humans has been identified. A recently developed immunosuppressive drug which might fulfil such a role is mycophenolate mofetil (MMF).

MMF is an immunosuppressant currently undergoing intensive clinical assessment. It is the morpholinooethyl ester of mycophenolic acid and inhibits de novo purine biosynthesis [1]. The theoretical advantage of MMF over conventional immunosuppressants is that this inhibition is thought to be relatively specific to lymphocytes, as other cell types have alternative pathways of purine synthesis which are not blocked by MMF [2]. MMF has been clearly demonstrated to impair T cell immunity in both experimental systems and in humans. The prevention of graft rejection by MMF has also been demonstrated in a number of animal models [reviewed 2,3]. MMF has a significant effect on T cell-mediated processes in humans. Clinical trials have shown that MMF prevents episodes of acute rejection in transplant recipients [e.g. 4–7] and reduces the severity of rheumatoid arthritis [8].

It would be anticipated that MMF might suppress the T cell-dependent humoral immune response to some extent via its effect on T cells. Animal studies, however, suggest that the effect of MMF on antibody production may be more profound than predicted by suppression of T cell help alone. In mice, MMF virtually abolishes the humoral response to sheep red blood cells [9]. The production of natural xenoreactive anti-
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Subjects and methods

Study population

Thirty-eight consecutive patients who had received renal transplants at the Royal Melbourne Hospital up to September 1993 and who had stable renal function, received influenza vaccination in 1994 as recommended by the National Health and Medical Research Council, Canberra. Twenty-five were treated with a regimen of prednisolone, cyclosporine and azathioprine (PCA group) while in the remaining 13 the azathioprine was replaced by MMF (PCM group). Doses of immunosuppressives were as detailed in Table 1. Patients were treated with either 2 g or 3 g of MMF daily in divided doses and were randomly allocated to these doses as part of the Tricontinental study [6]. All patients in the PCM group and seven patients in the PCA group were enrolled in the Tricontinental study, and the remaining patients in the PCA group comprised the 18 consecutive patients transplanted after the Tricontinental study’s recruitment period. In addition, 20 hospital staff members were recruited as normal controls. All those approached agreed to participate in the study, giving informed consent in accordance with the guidelines of the Board of Medical Research/Royal Melbourne Hospital Ethics Committee.

Influenza vaccine

Each subject was vaccinated in an 8-week period in late Autumn 1994 with a 0.5 ml intramuscular injection of influenza vaccine drawn from a single batch (Fluvax: kindly provided by CSL Limited, Parkville, Australia). Fluvax is a split virion influenza vaccine and the 1994 formulation contained 15 μg/0.5 ml dose of the haemagglutinins of A/Texas/36/9-like, A/Beijing/32/92-like and B/Panama 45/90-like strains.

Immunization regimen

Serum was collected on the day of vaccination from all subjects and again 4–6 weeks later. Those who had a titre of <40 to one or more antigens after this time received a second dose of vaccine. Serum samples were taken before and 4–6 weeks after booster vaccination.

Serology

Sera were tested for total specific antibody to influenza antigens A/Texas/36/9 (H1N1), A/Beijing/32/92 (H3N2) and B/Panama 45/90 by microtitre haemagglutination using a standard method [17]. Influenza antigens for use in antibody tests were supplied by the WHO Collaborating Centre for Influenza, CSL Limited. Sera were stored at −70°C until all samples had been collected, then were analysed in parallel in a blinded fashion. Titres were expressed as the reciprocal of the dilution showing 100% haemagglutination by 4 haemagglutinating units of virus.

Statistical analysis

Geometric mean titre was calculated after assigning titres of <1/20 an arbitrary value of 1/10, as is conventional in such analyses [e.g. 18]. Differences between baseline and post-immunization titres were analysed using the Wilcoxon matched pair test. Differences between groups were analysed using ANOVA, Fisher’s Exact Test or the Mann–Whitney test as indicated.

Results

No variables differed significantly between the transplant patient groups (Table 1). The age, creatinine and urea concentrations did differ between the control and both transplant groups (ANOVA). The differences were small, however, and would be considered unlikely to cause a significant difference in the immune response [16]. That the two transplant groups had similar renal function is of particular importance, as significant renal impairment causes a similar degree of suppression of the response to influenza vaccine as cyclosporine [16]. No episodes of rejection occurred during the period of the study or in the 2 months following it, and the only adverse reaction to vaccination was local erythema, pain and fever occurring in one member of the control group.

Studies of influenza vaccination have traditionally used one of two serological indicators to determine if a clinically effective immune response has occurred. One is the achievement of a 4-fold or greater rise in antibody titre to each specific antigen, irrespective of the titre at baseline [15,16]. In the control group a 4-fold rise in titre to 50% of immunizing antigens was seen. Treatment with PCA was associated with a lesser proportion (33%), consistent with previous observations [16]. The response in the PCM group was further
Table 1. Characteristics of subject groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>PCA</th>
<th>PCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td>male:female</td>
<td>7:13</td>
<td>15:10</td>
<td>5:8</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>35.6±2.3</td>
<td>40.8±2.6</td>
<td>48.0±3.0</td>
</tr>
<tr>
<td>Creatinine (mmol/l)*§</td>
<td>0.08±0.003</td>
<td>0.15±0.01</td>
<td>0.12±0.01</td>
</tr>
<tr>
<td>Urea (mmol/l)*§</td>
<td>5.2±0.3</td>
<td>11.1±0.9</td>
<td>9.7±1.0</td>
</tr>
<tr>
<td>Months from transplant to 1st vaccination*</td>
<td>3.1–7.3</td>
<td>6.1–22.6</td>
<td>5.8–18.9</td>
</tr>
</tbody>
</table>

| No. receiving OKT3 (%) | 8 (32%) | 1 (7.6%) |
| Rejection Episodes per patient* | 0–3 | 0–3 |
| Prednisolone dose (mg/day)* | 7.1±0.7 | 7.5±0.6 |
| Cyclosporine dose (mg/day)* | 230.0±16.1 | 227±12.2 |

*Values given as mean±SEM, followed by range.
§At time of first vaccination.

Reduced, with a 4-fold rise in titre occurring to only 10% of antigens (Figure 1A). The proportion of patients failing to mount a 4-fold rise in titre to any of the three antigens was considerably higher in the PCM group (Figure 1B). The other method used to determine the effectiveness of influenza vaccination is to assess conversion from a ‘non-protective’ (<40) to a ‘protective’ (≥40) titre after immunization [18,19]. Such an assessment confirmed the above findings (Figure 2A). No patient on MMF responded to all three antigens and many failed to respond to any at all, in contrast to the control and PCA groups (Figure 2B and C). Finally, the geometric mean titre of antibody against each antigen was increased by immunization in both the control and PCA groups, although this increase was more marked in the former group. In contrast, immunization failed to produce a significant rise in geometric mean titre to any antigen in the PCM group (Table 2). The effect of MMF seemed to be equivalent when either 2 or 3 g of MMF was administered, though the numbers treated with each dose were too small to allow a statistical comparison to be made. Thus the substitution of MMF for azathioprine was associated with a suppressed humoral response to influenza vaccination and above the previously noted suppressive effect of cyclosporine.

Pre-vaccination titres did not differ significantly between groups, though there was a tendency for them to be lower in the immunosuppressed groups (with the exception of the titre to influenza B antigen in the PCM group, discussed below) (Table 2). Similarly, the proportion of patients with a titre of ≥40 before vaccination seemed lower in the immunosuppressed groups, though this did not reach statistical significance (one way ANOVA). The proportions with such a titre were: AH1N1: control 40%, PCA 24%, PCM 7.6%; AH3N2: control 10%, PCA 0%, PCM 0%; and B: control 10%, PCA 12%, PCM 30%. These differences are consistent with previous observations that MMF reduces serum antibody levels in general, for example reducing levels of both total immunoglobulin and rheumatoid factor in patients treated for rheumatoid arthritis [8]. Any effect of differences in pre-immune titres on the outcome of the study were minimized by analysing the data in three different ways: looking at mean titres (Table 2), rise in titre in those with a pre-immune titre <40 (thus excluding those with a ‘protective’ pre-immune titre; Figure 2) and at those mounting a ≥4-fold rise in titre (irrespective of pre-immune titre; Figure 1). In addition, the presence of a high pre-vaccination antibody titre might be expected to bind to and neutralize administered antigen and reduce the subsequent response, which would tend to lead to underestimation rather than exaggeration of the findings of this study.

The effect of booster immunization in 24 subjects (seven control, nine PCA and eight PCM patients) not responding to one or more antigens was examined. The cumulative number of antigens with an antibody titre of <40 pre-boost was 46. Among those 46, only six (13%) converted to a protective titre of ≥40 after a second immunization, with no significant difference observed between the three groups.

Fig. 1. Response to influenza vaccination: ≥4-fold rise in titre. A The proportion of patients demonstrating a ≥4-fold rise in titre to each of the three antigens and all antigens. B The proportion of patients failing to mount a ≥4-fold rise in titre to any of the three antigens. These are 25% (5/20 patients), 36% (9/25) and 76% (10/13) for the control, PCA and PCM groups, respectively. Open, grey and black bars represent the control, PCA and PCM groups, respectively. *P<0.05, **P<0.01, ***P<0.001 (A, Mann–Whitney test; B, Fisher’s Exact Test).
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Fig. 2. Response to influenza vaccination: change from 'non-protective' (<40) to 'protective' (≥40) titre. A Proportion (%) of patients changing from a non-protective (<40) to protective (≥40) titre after vaccination, of those with titres <40 before immunization. The patients with a titre of ≥40 to each antigen prior to immunization were distributed as follows: AH1N1: control 40%, PCA 24%, PCM 7.6%; AH3N2: control 10%, PCA 0%, PCM 0%; and B: control 10%, PCA 12%, PCM 30%. Open, grey and black bars represent the control, PCA and PCM groups, respectively. B and C Proportion (%) of all patients with either a protective (≥40) or non-protective (<40) titre to all three antigens after immunization. *P<0.05; **P<0.01; ***P<0.001 (A: Mann-Whitney test; B and C, Fisher's Exact Test).

Table 2. Antibody response to influenza vaccination

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Control</th>
<th>PCA</th>
<th>PCM</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>AH1N1</td>
<td>25.9</td>
<td>134.5*</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>(&lt;20-80)</td>
<td>(20-320)</td>
<td>(&lt;20-20)</td>
</tr>
<tr>
<td>AH3N2</td>
<td>13.2</td>
<td>52.8*</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>(&lt;20-80)</td>
<td>(20-320)</td>
<td>(&lt;20-20)</td>
</tr>
<tr>
<td>B</td>
<td>15.2</td>
<td>47.6*</td>
<td>16.0</td>
</tr>
</tbody>
</table>

Values given are geometric means of titres followed by the range in brackets. The Wilcoxon matched pair test was used to compare pre- and post-vaccination values within each group (*P<0.0005; *no significant difference). Comparisons between the groups before vaccination did not show significant differences, except that the PCM anti-AH1N1 titre was lower than control (P=0.01, Mann–Whitney test). After vaccination, the titre against AH1N1 was less in PCM than either PCA (P=0.0007) or control (P<0.0001), and against AH3N2 both PCM and PCA groups were less than control (P=0.0007 and 0.009, respectively).

Discussion

We have observed marked suppression of the antibody response to influenza vaccination in renal transplant patients treated with MMF, indicating that MMF in combination with cyclosporine produces a greater suppression of the humoral immune response than conventional immunosuppressive regimens. This finding is supported by recent work showing a reduced titre of anti-ATGAM antibodies in patients treated with MMF [20].

The response to influenza B antigen did not appear to be as completely suppressed by MMF as those to the A antigens in the vaccine. This impression is unlikely to be of significance. There was no significant rise in mean titre to the B antigen after vaccination in the PCM group, in contrast to the PCA and control groups (Table 2). The reduction in patients mounting a >4-fold rise in titre appeared reduced to a similar degree in the PCM and PCA groups, though neither reduction reached statistical significance (Figure 1A). This result might have been influenced by the proportion of patients with high pre-immune titres which might make the achievement of a 4-fold rise in titre less likely. Similarly, the reduction in those converting from a non-protective to protective titre appeared equal in the PCM and PCA groups but neither reached statistical significance (Figure 2A). Thus the power of this study did not allow statistically useful analysis of the response to the B antigen in either PCA or PCM groups, though the trend was for suppression of the response in both groups to at least the same extent and, at least when considering mean titres, this suppression was significantly greater in the PCM group. Nonetheless, it remains a possibility that MMF does not reduce B cell responses to all antigens equally, which should be kept in mind when planning treatment strategies.

The suppression of B cell immune responses by MMF has a number of implications. Firstly, patients treated with MMF may be at greater risk of infection than others. Thus far this has not been borne out in published trials of MMF use [e.g. 4–8], perhaps reflecting the relative importance of cell-mediated compared to humoral immunity in defence against the predominantly viral infections which occur soon after transplantation. Nonetheless, long term follow-up of...
patients treated with MMF to determine whether they have increased rates of infection with organisms whose efficient clearance is dependent on antibody production, such as encapsulated bacteria, seems warranted.

These results also suggest that routine vaccination of transplant patients receiving MMF is likely to be ineffective. This may be true for vaccinations besides influenza, and the effect of MMF on other vaccine responses (e.g. hepatitis B, pneumococcus vaccine) should be investigated. This emphasizes the potential importance of vaccinating hospital staff and perhaps family members of immunosuppressed patients, as reduction in transmission of the virus to patients may be more protective than vaccinating the patients themselves. Vaccination before transplantation when possible would have obvious advantages. Booster vaccination was not sufficiently effective to warrant routine measurement of vaccine response to direct its use.

The fact that MMF profoundly reduces the humoral immune response to foreign antigens, over and above that suppression afforded by more conventional immunosuppressive regimes, may also have a more broad clinical application. For example, MMF may be particularly effective in treating diseases mediated by antibodies, such as systemic lupus erythematosus, IgA nephropathy, Goodpasture’s syndrome, myasthenia gravis and bullous pemphigoid. Recent case reports support this proposition [21,22]. In treating such diseases MMF, used alone or in combination with other immunosuppressives, may be more effective than regimens which are less specifically directed at antibody production. The use of MMF might have the potential to improve therapeutic outcome and allow a reduction in the dose, and therefore toxicity, of less selective immunosuppressants.

In conclusion, this study has demonstrated a marked suppression of the antibody response to influenza vaccination in renal transplant recipients treated with MMF, which has implications for the design of immunization protocols to protect the immunosuppressed. More importantly, the relatively specific effect of MMF on antibody production in humans may well be far more useful in treating antibody-mediated diseases than conventional immunosuppressive drugs.

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References

3. Allison AC and Eugui EM. Immunosuppressive effects of mycophenolic acid and an ester prodrug, mycophenolate mofetil. Imm Rev 1993; 136: 5–28