Immunoglobulin G (IgG) Subclass Distribution and IgG1 Avidity of Antibodies in Human Immunodeficiency Virus-Infected Individuals after Revaccination with Tetanus Toxoid

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In human immunodeficiency virus (HIV)-infected individuals the amount of antibodies formed after vaccination with T-cell-dependent recall antigens such as tetanus toxoid is proportional to the peripheral blood CD4+ T-lymphocyte counts. To investigate whether the immunoglobulin G (IgG) subclass distribution and avidity of the antibodies produced after vaccination are affected as well, we gave 13 HIV-infected adults with low CD4+ T-lymphocyte counts (<200 × 10^3/liter; group I), 11 HIV-infected adults with intermediate CD4+ T-lymphocyte counts (≥200 × 10^3/liter; group II), and 5 healthy controls booster immunizations with tetanus toxoid. The prevaccination antibody concentrations against tetanus toxoid were similar in the HIV-infected and healthy adults. After vaccination the total IgG and the IgG1 anti-tetanus toxoid antibody concentrations were significantly lower in group I than in group II and the controls. The avidity of the IgG1 anti-tetanus toxoid antibodies formed by HIV-infected adults was within the range for healthy controls, irrespective of their CD4+ T-lymphocyte counts.

For the present study we selected 24 HIV-infected individuals and 5 healthy controls from the population from the study described above. Informed consent was obtained from all individuals. The criteria for inclusion were a prevaccination anti-tetanus toxoid IgG antibody concentration of ≤0.01 arbitrary units/ml (≤0.05 μg/ml) and the ability to mount a humoral response to tetanus toxoid, i.e., a ≥1.25-fold increase in the IgG anti-tetanus toxoid antibody concentration after revaccination (4). Thirteen of 27 HIV-infected individuals with peripheral blood CD4+ T-lymphocyte counts of <200 × 10^3/liter (group I) and 11 of 21 HIV-infected individuals with ≥200 × 10^3 CD4+ T-lymphocytes/liter (group II) fulfilled the criteria of selection. These individuals did not differ from the nonselected individuals concerning clinical or laboratory parameters, e.g., CD4+ T-lymphocyte counts. Patient characteristics are presented in Table 1. In the sera from the selected individuals described above, total IgG, IgG subclasses, and IgA anti-tetanus toxoid antibodies were quantified by an antibody-capture enzyme-linked immunosorbent assay (ELISA) (6). In short, the wells of a 96-well polystyrene microtiter plate were coated with tetanus toxoid, blocked with bovine serum albumin, and incubated with twofold serial dilutions of serum samples and standard sera. Total IgG and IgA anti-tetanus toxoid antibodies were measured by the addition of alkaline phosphatase-conjugated goat anti-human IgG (γ-chain specific) and goat anti-human IgA (α-chain specific), respectively (Tago, Burlingame, Calif.). Antibodies in the IgG subclasses were measured by successive incubation with IgG subclass-specific monoclonal antibodies (anti-IgG1, MH 161-1 [CLB, Amsterdam, The Netherlands]; anti-IgG2, 51-1-27 [TNO, Leiden, The Netherlands]; anti-IgG3, NI 86 [Nordic, Tilburg, The Netherlands]; anti-IgG4, NI 315 [Nordic]), followed by incubation with alkaline phosphatase-conjugated rabbit anti-mouse Ig (Dakopatts, Glostrup, Denmark). After incubation with substrate (p-nitrophenylphosphate), the reaction was stopped with 3 M NaOH, and the optical density at 405 nm was recorded with a Titertek Multiscan (Labsystems, Helsinki, Finland). Based on the standard sera containing known amounts of anti-tetanus toxoid antibodies, a reference curve was constructed. Use of this curve allowed the calculation of the amount of anti-tetanus toxoid antibodies of the respective classes or subclasses in the sera of the patients and the controls. The avidity of IgG1 anti-tetanus toxoid was measured by a modified elution ELISA, in which well-chosen dilutions of serum samples were allowed to interact with tetanus toxoid coated on the wells of microtiter plates (9). For each of the serum samples, dilutions containing 50 and 25% of the amount of the anti-tetanus toxoid antibodies which can maximally bind to the coated antigen were chosen. Thereafter, the wells were incubated with a variable molarity (range, 0.5 to 4.5 M) of the chaotropic agent sodium thiocyanate (NaSCN). IgG1 anti-tetanus toxoid antibody concentrations were then measured as described above. The relative avidity index is defined as the molarity of NaSCN at which 50% of the amount of IgG1 subclass antibodies that are bound to the coated...
tetanus toxoid in the absence of NaSCN has been eluted from the antigen. Concentrations of antibody and avidity indexes were log transformed to correct for skewness in the distribution. A multiple-comparison procedure was used to determine whether mean values were significantly different between groups (P, 0.05; Bonferroni-adjusted t test).

**RESULTS**

Before vaccination, the geometric mean concentrations of total IgG, the IgG subclasses, and IgA antibody against tetanus toxoid were similar in groups I and II and were comparable to those in the healthy controls (Table 2). After vaccination the total IgG anti-tetanus toxoid antibody concentration was significantly lower in group I compared with those in group II and the controls. The IgG anti-tetanus toxoid response after booster vaccination of HIV-infected individuals and healthy controls consisted predominantly (≥75%) of antibodies of the IgG1 subclass (Table 2). Consequently, the production of IgG1 was significantly reduced in group I. The postvaccination geometric mean concentrations of IgG2 and IgG3 anti-tetanus toxoid antibodies were significantly lower in group I than in group II (Table 2). In all groups of patients and controls a significant increase in IgG and IgG1 anti-tetanus toxoid antibodies was induced by vaccination (paired t test of logarithmically normalized values) (Table 2; Fig. 1).

Since the IgG1 anti-tetanus toxoid antibody is quantitatively the major IgG subclass formed after vaccination with tetanus toxoid, the avidity of IgG1 antibodies was investigated. In the HIV-infected individuals with low CD4+ T-lymphocyte counts (group I) and with low IgG1 anti-tetanus toxoid antibody concentrations after vaccination, the mean avidity of the IgG1 anti-tetanus toxoid antibodies was similar to the mean avidity of the IgG1 anti-tetanus toxoid in individuals in group II and in healthy controls (P, 0.2; Bonferroni-adjusted t test) (Fig. 2).

The IgG1 antibodies from three patients with CD4+ T-lymphocyte counts of 40 × 10⁶, 40 × 10⁶, and 105 × 10⁶ cells/liter, respectively, demonstrated a significant increase in avidity after vaccination.
TABLE 2. Anti-tetanus-toxoid antibody concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Total IgG</th>
<th>IgG3</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG1</th>
<th>IgG2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geometric mean (range) concn. (μg/ml)</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>FI</td>
<td>4.41 (1.15-5.9)</td>
<td>2.77 (1.7-2.9)</td>
<td>2.0 (0.6-4.0)</td>
<td>3.3 (1.1-12.2)</td>
<td>5.6 (1.8-4.2)</td>
<td>1.0 (0.5-3.1)</td>
<td>1.2 (0.7-1.4)</td>
<td>0.7 (0.4-1.2)</td>
<td>3.5 (1.3-5.5)</td>
<td>5.6 (1.8-4.2)</td>
<td>1.0 (0.5-3.1)</td>
<td>1.2 (0.7-1.4)</td>
<td>0.7 (0.4-1.2)</td>
<td>3.5 (1.3-5.5)</td>
<td>5.6 (1.8-4.2)</td>
<td>1.0 (0.5-3.1)</td>
</tr>
<tr>
<td>C</td>
<td>4.10 (1.2-2.6)</td>
<td>4.10 (1.2-2.6)</td>
<td>4.10 (1.2-2.6)</td>
<td>4.10 (1.2-2.6)</td>
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<td>4.10 (1.2-2.6)</td>
<td>4.10 (1.2-2.6)</td>
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</table>

DISCUSSION

In earlier studies, we have demonstrated that the formation of IgG anti-tetanus toxoid antibodies after vaccination is impaired in HIV-infected adults with CD4+ T-lymphocyte counts below 300×10^6/liter (7, 8). The results of the present study indicate that the IgG antibodies formed after booster vaccination with tetanus toxoid are predominantly of the IgG1 subclass, both in healthy controls and in HIV-infected individuals. This is compatible with the fact that tetanus toxoid, being a protein antigen, induces a T-cell-dependent immune response which consists mainly of IgG1 in healthy adults (2, 11) as well as in HIV-infected individuals (1). The present study extends our earlier observations and demonstrates that HIV-infected individuals with fewer than 200 CD4+ T lymphocytes exhibit a quantitatively impaired IgG1 anti-tetanus toxoid response. The finding of a normal total IgG anti-tetanus toxoid response, and consequently, a normal IgG1 antibody response, in the HIV-infected individuals with high CD4+ T-lymphocyte counts (group II) confirms the observations of Ballet et al. (1).

Since we found normal tetanus toxoid-specific IgG1 avidity indices even in individuals with low CD4+ T-lymphocyte counts and with low IgG1 anti-tetanus toxoid antibody concentrations, we may conclude that the functional avidity of the formed antibodies is optimal. Janoff et al. (5) have described similar results in HIV-infected individuals by studying the avidity of total IgG anti-tetanus toxoid antibodies.

After repeated vaccination the affinity of antibodies gradually increases. This affinity maturation is a consequence of mutation of the immunoglobulin genes followed by selection of B-cell clones. Such a production of memory B cells, which occurs only in T-cell-dependent antibody responses, confers a long-lasting ability to respond to subsequent encounters with the same antigen (10). A booster vaccination with T-cell-dependent antigens involves activation of memory B cells, which requires less antigen and fewer CD4+ T lymphocytes than the activation of unprimed B cells (13). Although HIV-infected individuals with low CD4+ T-lymphocyte counts produce less anti-tetanus toxoid antibody after booster vaccination, the memory B lymphocytes, generated during an earlier vaccination, presumably before exposure to HIV, still appear to be effectively triggered and the avidity of the IgG1 anti-tetanus toxoid antibody that is produced is not affected. Interesting in this respect is the observation of the increase in avidity induced by vaccination in three patients with low CD4+ T-lymphocyte counts and low IgG1 anti-tetanus toxoid antibody levels, indicating that somatic hypermutation leading to avidity maturation can still be induced in residual memory B-lymphocyte clones, despite the low numbers of T helper cells.

The results of this study indicate that HIV-infected individuals with low CD4+ T-lymphocyte counts mount a qualitatively decreased but qualitatively normal antibody response to tetanus toxoid revaccination. Therefore, even in this group of HIV-infected individuals, vaccination with a T-cell-dependent recall antigen appears to result in the induction of an antibody response which may be protective.
REFERENCES