In this observational study, mumps-specific in vitro lymphoproliferation was measured in 24 subjects with low antibody titers and 24 subjects with high antibody titers who received their last vaccine dose up to 16 years previously. Overall, a significant lymphoproliferative response was found in 32 subjects (66.7%)—namely, in 13 (54.2%) of those with low antibody titers and 19 (79.2%) of those with high antibody titers. The mean stimulation index for subjects with low antibody titers was 4.47, whereas that for subjects with high antibody titers was 8.31 (P = .032). Mumps vaccine–induced cell-mediated immunity appears to be more persistent than the antibody response.

In countries with long-standing, national, 2-dose measles, mumps, and rubella (MMR) vaccination programs, the incidence of mumps and the rate of associated complications have been significantly reduced [1]. In 2006, however, a large outbreak of mumps occurred in the United States, in which 67% of all affected individuals had received 2 doses of MMR vaccine [2].

In Belgium, it is recommended that children receive 2 MMR doses, the first of which is suggested to be given at 15 months of age (since 1985) and the second at 10–12 years of age (since 1995). From 1985 until 1993, the Urabe strain was used, and, since that time, the Jeryl Lynn strain has been administered. After the initiation of the MMR vaccine program in Belgium, the incidence of mumps decreased rapidly, and the current prevalence of mumps has been low, with <1 case reported per 100,000 inhabitants annually over the past few years (V. Van Casteren, written communication, 2001, and T. Lernout, written communication, 2006).

Prelicensure studies of the Jeryl Lynn and Urabe mumps vaccines in seronegative children showed high seroconversion rates (~95%) after vaccination [1]. Despite the initial success observed, several studies have shown that antibodies induced with 1 dose of vaccine tend to wane over time, and this decrease coincides with mumps outbreaks among children in primary and secondary schools [3]. Two recent studies also demonstrated a waning of antibodies in subjects who received 2 doses, the second of which was given 15 years earlier [4, 5].

Like measles and rubella vaccines, mumps vaccines also induce cellular immunity in addition to the humoral immune response. Until recently, this aspect of mumps vaccination has been poorly examined. In 2001, Gans et al. [6] showed that the mumps vaccine is capable of inducing a lymphoproliferative response in infants vaccinated at 6, 9, or 12 months of age. Recent studies of the long-term persistence of cell-mediated immunity showed that mumps-specific lymphoproliferative responses are still present in 70%–98% of adults given 2 doses of vaccine with mumps component, irrespective of the presence or absence of circulating anti-mumps antibodies [7–9]. The recent outbreak in the United States (in which most affected individuals had received 2 vaccine doses) raises the question of whether memory T cells are able or sufficient to prevent mumps outbreaks in the absence of antibodies.

To extend our insight regarding the persistence of mumps-specific T cell responses induced by 1 or 2 vaccine doses given up to 16 years previously, we compared the mumps-specific lymphoproliferative responses in groups of vaccine recipients with low and high levels of anti-mumps antibody.
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Table 1. Demographic and vaccination characteristics and lymphoproliferative responses noted in both study groups, according to anti-mumps antibody (Ab) status.

<table>
<thead>
<tr>
<th>Finding</th>
<th>Low Ab titer (n = 24)</th>
<th>High Ab titer (n = 24)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex ratio, no. of males: no. of females</td>
<td>3:21</td>
<td>3:21</td>
<td>.28</td>
</tr>
<tr>
<td>Age at blood sampling, mean ± SD, years</td>
<td>20.2 ± 1.0</td>
<td>19.8 ± 1.0</td>
<td>.28</td>
</tr>
<tr>
<td>Age at vaccination, mean ± SD (range), years</td>
<td>First dose</td>
<td>3.7 ± 4.4 (0.9–18.8)</td>
<td>.28</td>
</tr>
<tr>
<td></td>
<td>Second dose</td>
<td>11.8 ± 1.4 (9.4–14.5)</td>
<td>.53</td>
</tr>
<tr>
<td>Interval to sample collection, mean ± SD, years</td>
<td>From receipt of only vaccine dose</td>
<td>16.7 ± 4.6</td>
<td>.93</td>
</tr>
<tr>
<td></td>
<td>From receipt of second vaccine dose</td>
<td>7.8 ± 1.6</td>
<td>.71</td>
</tr>
<tr>
<td>SI</td>
<td>&lt;3 (n = 16)</td>
<td>11 (45.8%)</td>
<td>.032</td>
</tr>
<tr>
<td></td>
<td>&gt;3 (n = 32)</td>
<td>13 (54.2%)</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>4.47 ± 4.68</td>
<td>8.31 ± 7.05</td>
<td></td>
</tr>
</tbody>
</table>

a Titer <230.

b Titer ≥500.

c Sixteen of these subjects had a low Ab titer, and 10 had a high Ab titer.

d Eight of these subjects had a low Ab titer, and 14 had a high Ab titer.

e The data for measurement of lymphoproliferative responses are expressed as the no. and the fraction (%) of subjects displaying a stimulation index (SI) ≥3.

f As determined by Fisher’s exact test for the lymphoproliferation assay, according to humoral immunity (P = .12).

null
the difference did not reach statistical significance (P = .12). The mean SI for the group with low antibody titers was significantly lower than that for the group with high antibody titers (4.47 vs. 8.31; P = .032) (figure 1). When the lymphoproliferation assay (LPA) response was compared according to the number of doses of vaccine containing mumps virus, 16 (61.5%) of 26 subjects who received only 1 vaccine dose and for whom the mean interval to serum sample collection was 16 years still had a positive LPA response, whereas 16 (72.7%) of 22 subjects who received 2 doses of vaccine still had a SI ≥3. However, the outcome of the LPA was not influenced either by the number of doses of mumps vaccine virus received (P = .96) or by the interval since the last vaccination (P = .47).

All subjects, irrespective of their mumps antibody titer, displayed a positive lymphoproliferative response (SI, ≥3) toward TT and VZV. This finding indicates that the PBMCs were of good quality and could mount a strong lymphoproliferative response to in vitro antigens and that the observed differences in the in vitro responses to mumps virus antigen were indeed associated with the differences in mumps antibody titers.

Discussion. Mumps-specific lymphoproliferative responses can be elicited in vitro up to 16 years after the administration of 1 or 2 doses of mumps vaccine virus, even in subjects who have either no or low anti-mumps antibody levels left at that time. Subjects with high levels of antibodies display positive lymphoproliferative responses (SI, ≥3) more frequently than do subjects with no or low antibody levels (19 [79.2%] of 24 subjects vs. 13 [54.2%] of 24 subjects, respectively) (table 1), and these responses are of a higher magnitude (geometric mean SI, 8.31 vs. 4.47, respectively) (figure 1).

These observations, along with the recent mumps outbreak in the United States, raise a question about the extent to which persistent cellular immunity contributes to the long-term protection against mumps virus infection and disease. Is cellular immunity capable of effectively protecting individuals against mumps in the absence of anti-mumps antibodies? In humans, protection against mumps is primarily based on the generation of mumps-specific antibodies. This, of course, requires the stimulation, expansion, and maturation of antigen-specific B cells, processes that need adequate support from CD4+ T helper lymphocytes. It has been shown that the T cell immune system plays an essential role in the establishment of high antibody titers, as well as the elimination of virus in instances of natural infection [11].

Sustained antibody-mediated protection largely relies on long-lived IgG-producing plasma cells and memory B cells [11, 12]. When antibodies wane or even disappear because of a lack of recurrent exposure to wild-type virus or revaccination, protection will depend on the speed with which the immune system is able to mount a protective antibody response and prevent the virus from invading the human host. For tetanus and diphtheria, it has been shown that circulating antibodies are crucial in the prevention of disease, whereas this seems to be less important for the hepatitis B virus, given its long incubation period [12].

Because we and other investigators have demonstrated that cellular immune responses to the mumps virus can be identified in the majority of vaccinated individuals, irrespective of their anti-mumps antibody status, outbreaks such as the one experienced in the United States in 2006 suggest that circulating anti-
bodies are the most important defense mechanism against mumps. Experience with the Rubini mumps vaccine strain further supports this view. The Rubini mumps vaccine strain was capable of inducing strong lymphoproliferative responses in individuals who were vaccinated twice, whereas the antibody responses were rather weak. The numerous outbreaks that occurred in populations vaccinated with this strain suggest that immune protection against mumps is more dependent on high antibody titers than on cellular immune responses [13, 14].

Moreover, in the present study, we show that 61.5% of the recipients of 1 vaccine dose still had positive lymphoproliferative responses, despite the fact that they were vaccinated up to 16 years previously. Several studies of outbreaks have shown that the risk of developing mumps after receipt of only 1 dose of mumps vaccine virus increases with time [1, 3]. If cellular immunity in the absence of circulating wild-type mumps virus would protect against disease, the likelihood of outbreaks developing after 1 dose would be low.

Overall, these findings emphasize the importance of circulating antibodies in the protection against mumps disease. Should we rethink the vaccination program for mumps? In the latest US outbreak, the effectiveness of the 2-dose vaccine schedule was estimated to be 76%–88%, and no differences were observed between the attack rates noted for recipients of 1 and 2 vaccine doses. This vaccine effectiveness is similar to previous estimates of the efficacy of 1 vaccine dose [15]. Moreover, in a Finnish longitudinal study, 74% of the vaccine recipients who initially were seronegative still had detectable antibodies against mumps 15 years after receiving a second dose, but only 40% showed seroprotective titers in the ELISA that was also used in the present study. This study clearly shows a decrease in the proportion of individuals with high antibody titers, despite the administration of 2 doses. The pool of susceptible adolescents and adults will increase annually, and this may jeopardize the elimination of mumps that has been achieved in such countries as Finland [4]. If the persisting cellular immunity offers no or insufficient protection in the absence of circulating antibodies, than mumps vaccination policies should be reevaluated at the level of timing of and the number of doses to be administered.

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References