Maternal Monoclonal Antibody to the V3 Loop Alters Specificity of the Response to a Human Immunodeficiency Virus Vaccine

Marie T. Jelonek,* Jennifer L. Maskrey, Kathelyn S. Steimer, Barbara J. Potts,* Keith W. Higgins, and Margaret A. Keller

The effect of maternally transferred monoclonal antibody (MAb) on the offspring antibody response to rgp120$_{SF2}$ was examined in a murine model. Two MAbs were studied: MAb 83.1, which recognizes a determinant in the V3 loop of gp120 from human immunodeficiency virus-1 (HIV-1) SF2, and MAb 26.2D3, which recognizes a conserved N-terminal region of gp120 from HIV-1$_{SF2}$. Offspring were immunized at 18–21 days of age with 100 μg of rgp120$_{SF2}$ in complete Freund's adjuvant. Offspring immunized in the presence of preexisting MAb 83.1 but not MAb 26.2D3 demonstrated inhibition of the IgG anti-V3 response. The total IgG anti-rgp120$_{SF2}$ response was not affected by preexisting MAb. Since newborns at risk for HIV may be immunized in the presence of maternal or administered anti-HIV antibody, alternative strategies may be required to circumvent inhibition of the infant's epitope-specific response to HIV immunization by preexisting antibody.

Many strategies are being evaluated to prevent transmission of human immunodeficiency virus (HIV) from an infected mother to her infant [1, 2]. The AIDS Clinical Trials Group (protocol 185) is currently evaluating the effect of HIV immune globulin on maternal transmission of HIV. Ultimately, antibody may be combined with a successful vaccine.

We have developed the murine model for studying effects of preexisting antibody on the murine offspring response to candidate HIV vaccines. In our earlier experiments, we found that maternally transferred polyclonal antibody profoundly inhibited the offspring IgG response to an rgp120 vaccine [3].

In experiments presented here, we demonstrate the effect of maternally transferred monoclonal antibody (MAb) on the subsequent offspring total antibody response and the fine specificity of that response.

Methods

Mice

Specific pathogen–free adult BALB/c mice (Jackson Laboratory, Bar Harbor, ME) were bred for not more than three generations in our laboratory. All immunizations and antibody injections were administered intraperitoneally (ip). MAb was administered to postpartum mice within 24 h after birth of the offspring in a total volume of 0.5–1.0 mL. Antigens were administered in complete Freund's adjuvant (CFA; Life Technologies GIBCO BRL, Gaithersburg, MD) in a total volume of 0.1–0.2 mL. Mice were bled through the retroorbital sinus.

MAb Administered to Postpartum Mice

MAb 83.1 (lot A-2; Repligen, Cambridge, MA) is a BALB/c IgG1 MAb that neutralizes HIV-1$_{SF2}$ [4]. This MAb binds to a determinant defined by amino acids IXIGPGR of the V3 loop of HIV-1 (X can be H, Y, T, R, N, S, or A). MAb 83.1 was prepared by immunization of mice with the Repligen peptide RP142 (YNKRKRFYTYKIGC), which is the V3 loop of HIV-

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Reprints or correspondence: Dr. Margaret A. Keller, Dept. of Pediatrics, Harbor-UCLA Medical Center, 1000 W. Carson St., Box 468, Torrance, CA 90509.
* Present affiliations: Molecular Biology Section, Laboratory of Immunology, National Institutes of Health, Bethesda, Maryland (M.T.); Tektagen, Malvern, Pennsylvania (B.J.P.).
HIV-1 sF2 [4]. Ninety percent neutralization of HIV-1 MN occurred to the V3-loop peptide RP150 (Repligen peptide 150) of HIV-1sF2.

Antigens Administered to Offspring Mice

ggp120sF2. Immunization was done ip with 100 μg of glycosylated recombinant gp120 from HIV-1sF2 in CFA. This vaccine (lot P1.6-a) was prepared at Chiron in CHO cells [5] and is currently used in clinical trials.

Hen egg white lysozyme (HEL). HEL is a 129–amino acid protein antigen that we have previously studied in the murine system [6, 7]. Immunization was also done ip with 100 μg of HEL in CFA. HEL was obtained from Societa Prodotti Antibiotici (Milan, Italy).

Experimental Design

The purpose of these experiments was to determine if preexisting maternally transferred MAb to the V3 loop of HIV-1sF2 would alter the magnitude and specificity of the offspring response to an rgp120sF2 vaccine. Three groups of offspring mice were studied: offspring of mice injected within 24 h postpartum with 500 μg of MAb 83.1, the anti–V3 loop MAb; offspring of mice injected postpartum with 500 μg of MAb 26.2D3, the control MAb; and control offspring of female mice without any antibody administered. All offspring mice were immunized ip at 18–21 days of age with 100 μg of rgp120sF2 in CFA. Each group consisted of 3 postpartum mice and the offspring.

In additional experiments, the offspring of 1 postpartum mouse injected with MAb 83.1 and the offspring of a control postpartum mouse were immunized at 18–21 days of age with HEL in CFA to assess nonspecific effects on the response to an unrelated protein antigen. These offspring mice were also used to determine the half-life of transferred maternal antibody (MAb 83.1) in the sera of the offspring.

Antibody Assays

Anti-rgp120sF2 IgG ELISA and anti-HEL IgG ELISA. We have described these methods in our earlier work [3, 7]. After incubation of dilutions of mouse sera on either rgp120sF2 or HEL-coated plates, alkaline phosphatase–conjugated goat anti-mouse IgG (Southern Biotechnology, Birmingham, AL) was used for development with p-nitrophenyl phosphate as substrate. Each assay was standardized with a pooled polyclonal serum obtained from adult mice immunized with rgp120sF2 in CFA or HEL in CFA. Each immune serum was assigned a value of 10,000 arbitrary units/mL and was used to generate a standard curve.

Anti-V3 (anti-RP150) IgG ELISA. Plates were coated as described [3] with RP150. This peptide represents the entire V3 loop of HIV-1sF2, and has the sequence NTRKS.IYI.GPGRAFHTTGRCI [4]. Alkaline phosphatase–conjugated goat anti-mouse IgG (Southern Biotechnology) was used for development with p-nitrophenyl phosphate as substrate. A standard curve was generated with MAb 83.1 (lots A-2 or 6).

Statistical Analyses

Statistical analyses used the BMDP (Los Angeles, CA) statistical software program. Probability values were calculated with the Fisher’s exact test and Student’s t test.

Results

Anti-rgp120sF2 IgG response. There was no effect of preexisting maternally transferred MAb on the total anti-rgp120sF2 IgG response to immunization. The total serum IgG antibody response was determined for 9 weeks after immunization of the offspring mice at age 18–21 days (table 1). The geometric mean titer at each interval after immunization was similar for offspring with preexisting MAb 83.1 or preexisting MAb 26.2D3 or for mice without preexisting antibody.

At the time of immunization (day 0), the serum geometric mean concentration of MAb 83.1 for offspring with preexisting MAb 83.1 was 8.1 μg/mL (95% confidence interval, 6.0–10.95 μg/mL). For offspring with preexisting MAb 26.2D3, the geometric mean concentration of 26.2D3 at the time of immunization was 2.84 μg/mL (95% confidence interval, 2.49–3.24 μg/mL). Thus, offspring with preexisting MAb 83.1 had ~3-fold increased preexisting MAb compared with offspring with preexisting MAb 26.2D3 on day 0 when both were assayed using rgp120sF2-coated ELISA plates.

Anti-V3 IgG antibody response. The fine specificity of the offspring vaccine response was altered by preexisting maternal MAb. Offspring mice, immunized in the presence of preexisting anti-V3 antibody, did not respond to the V3 epitope of rgp120sF2. The geometric mean titer of anti-V3 IgG antibody was significantly lower at both 42 and 63 days after immunization when the MAb 83.1 (V3-loop) group was compared to the non-MAb control group ($P < .001$; table 2). Similarly, the geometric mean titer of anti–V3 loop IgG antibody was significantly lower at both 42 and 53 days after immunization when the V3-loop MAb group was compared to the control MAb group ($P < .005$ and $P < .001$).

The concentration of preexisting anti-V3 loop antibody was 13.2 μg/mL in the V3-loop MAb group and was not detected in the control non-MAb group or the control MAb group. There was no effect of preexisting MAb 26.2D3, the control MAb, on the anti-V3 component of the offspring response compared with that of controls, presumably since this MAb recognizes an N-terminal determinant of rgp120sF2, not in the V3 loop.

The absence of the anti-V3 component is more striking when the data are examined as the number of mice in each group with detectable anti-V3 loop antibody. At 9 weeks after immunization (age 12 weeks), 18 of 20 non-MAb control mice and
17 of 23 mice in the control MAb group had detectable anti-V3 antibody. Only 2 of 19 mice in the MAb 83.1 group had detectable anti-V3 antibody 9 weeks after immunization. It was not possible to determine the source of the IgG antibody here with maternally transferred MAb did not demonstrate an inhibition of the total IgG anti-rgp120sF2 response but rather a profound alteration of the fine specificity of that response. In these MAb studies, preexisting MAb to the V3 loop of HIV-1 prevented an anti-V3 loop IgG antibody response following immunization with rgp120sF2. These results strongly support an epitope-blocking mechanism for the observed inhibition in both the previous polyclonal antibody and current MAb studies. If a more rapid clearance of antigen by preexisting antibody was the mechanism for the observed inhibition, the epitope-specific effect we observed would be very unlikely.

Many studies have examined the effects of preexisting antibody on the offspring response to immunization [8–12]. For the human infant, recent studies have demonstrated inhibitory effects of maternal antibody to tetanus toxoid [8], antibody to diphtheria toxoid [9, 10], and antibody to pertussis antigen [11]. Effects of maternal antibody on the murine offspring response to inactivated rabies virus vaccine have been studied by Xiang and Ertl [12]. Polyclonal maternal antibody from previously immunized mother mice inhibited the total antibody response of the offspring to vaccine as we also observed in our earlier studies [3]. These investigators also examined direct administration of a panel of MAbs. The fine specificity of the vaccine antibody response was examined, but no evidence of an epitope-specific inhibition was demonstrated. Our study demonstrating epitope-specific inhibition differed from the

### Table 1. IgG anti-rgp120sF2 response of offspring BALB/c mice immunized with rgp120sF2 in complete Freund’s adjuvant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 42</th>
<th>Day 63</th>
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</thead>
<tbody>
<tr>
<td>MAb 83.1</td>
<td>350 (295–415)</td>
<td>347 (244–493)</td>
<td>1025 (755–1393)</td>
<td>2650 (2077–3382)</td>
<td>5880 (4351–7947)</td>
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<tr>
<td>(n = 23)</td>
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<tr>
<td>MAb 26.2D3</td>
<td>700 (504–972)</td>
<td>763 (542–1073)</td>
<td>2250 (1682–3010)</td>
<td>4922 (3530–6864)</td>
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<tr>
<td>(n = 20)</td>
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<tr>
<td>No MAb</td>
<td>331 (236–465)</td>
<td>333 (181–610)</td>
<td>680 (337–1370)</td>
<td>2640 (2077–3382)</td>
<td>4866 (3737–6337)</td>
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<td>(n = 23)</td>
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NOTE: Postpartum mice were injected intraperitoneally with 500 µg of monoclonal antibody (MAb) within 24 h postpartum. Control mice were not injected. Offspring were immunized with 100 µg of rgp120sF2 intraperitoneally on day 0 (18–21 days of age). Each group contained mice from 3 litters. Data are geometric mean titer in ng/mL (95% confidence interval). --, no detectable antibody.

### Table 2. IgG anti-V3 loop response of offspring BALB/c mice immunized with rgp120sF2 in complete Freund’s adjuvant.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>MAb 83.1</td>
<td>13,210 (9590–18,200)</td>
<td>293 (196–438)</td>
<td>308 (244–388)</td>
<td>199* (156–254)</td>
<td>112* (89–141)</td>
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<tr>
<td>(n = 21)</td>
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<td>(n = 23)</td>
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<tr>
<td>No MAb (control)</td>
<td>209 (94–461)</td>
<td>407 (220–754)</td>
<td>1019 (578–1798)</td>
<td>786 (387–1599)</td>
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<tr>
<td>(n = 20)</td>
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NOTE: Postpartum mice were injected intraperitoneally with 500 µg of monoclonal antibody (MAb) within 24 h postpartum. Control mice were not injected. Offspring were immunized with 100 µg of rgp120sF2 intraperitoneally on day 0 (18–21 days of age). Data are geometric mean titer in ng/mL (95% confidence interval). --, no detectable antibody.

$P < .001$ compared with control mice, $^* < .01$ compared with MAb 26.2D3 mice, $^* < .001$ compared with MAb 26.2D3 mice.

Discussion

Our previous studies [3] have shown that maternal polyclonal antibody can profoundly suppress the murine offspring total IgG response to an rgp120sF2 vaccine. The studies presented here with maternally transferred MAb did not demonstrate an inhibition of the total IgG anti-rgp120sF2 response but rather a profound alteration of the fine specificity of that response. In these MAb studies, preexisting MAb to the V3 loop of HIV-1 prevented an anti-V3 loop IgG antibody response following immunization with rgp120sF2. These results strongly support an epitope-blocking mechanism for the observed inhibition in both the previous polyclonal antibody and current MAb studies. If a more rapid clearance of antigen by preexisting antibody was the mechanism for the observed inhibition, the epitope-specific effect we observed would be very unlikely.

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work of Xiang and Ertl [12] in that a single MAb was adminis-
terred, not a panel of MAbs with different fine specificities.

In addition to epitope masking, we have considered other
mechanisms of inhibition, including altered presentation of
peptides to T cells. Soluble antibody has been shown to block
B cell presentation of antigen to a T cell clone [13]. We cannot
exclude such a mechanism in our study. Our data also cannot
exclude idiotypic network perturbation in neonatal mice [14]
by maternal MAB since neonatal mice are very susceptible to
exposure to immunoglobulin idiotypes in the first weeks of life.
Since Fe-mediated inhibition of B cell responses is generally
considered to be antigen-specific, not epitope-specific, it is an
unlikely mechanism [15].

Our data suggest that epitope blocking has occurred in which
preexisting antibody to the V3 peptide prevented the recogni-
tion of this epitope by B cells. The studies of the catabolism
of MAB 83.1 in the offspring found a serum half-life of 12.3
days, suggesting that the anti-V3 antibody present in only 2
offspring, 9 weeks after immunization, may have been residual
maternal antibody. It is possible that none of the offspring
responded to the V3 peptide.

Although our studies have examined epitope-specific effects
of maternal antibody on the offspring response to vaccine anti-
gen, it is also possible that the specificity of the immune re-
sponse to natural infection with HIV-1 could be altered by
antibody. Such effects may be the result of maternal antibody
or administered neutralizing antibody during pregnancy or at
delivery. Altered specificity of the immune response to infec-
tion could potentially enhance the pathogenesis of HIV infec-
tion in the infant.

In summary, we have demonstrated that preexisting maternal
anti-V3 MAB, a neutralizing MAB for HIV-1, profoundly al-
terred the fine specificity of the offspring response to an
rgp120gp120 vaccine. Antibody to a neutralizing determinant, the
V3 loop, was missing from the response. Such studies clearly
demonstrate that preexisting antibody, either exogenously ad-
ministered directly to the infant or transferred to the infant
from the mother, may alter the vaccine response. HIV vaccines
that are successful in adults and older infants may not be suc-
cessful in the newborn. Alternative immunization strategies
may be necessary to overcome inhibition by maternal antibody
in the newborn.

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H. Smith for editorial review.

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