be recommended at the present time to discontinue therapy for patients who achieve a molecular remission with imatinib.

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References

To the editor:

**CCR5-specific mucosal IgA in saliva and genital fluids of HIV-exposed seronegative subjects**

CCR5 is a chemokine receptor expressed on blood T lymphocytes and monocyte-macrophages; in the genital tract, it works as the main HIV coreceptor.²⁻³ CCR5 mediates HIV entry following sexual transmission. Many studies have addressed the role of the CCR5 molecule as a putative target to prevent HIV infection. Serum antibodies to CCR5, found in subpopulations of HIV-exposed seronegative subjects (ESNs), have been considered to play a role in natural HIV resistance.⁴⁻⁵

We studied samples from 118 HIV-exposed subjects, sexual partners of HIV-seropositive patients (mean age, 34.7; range, 19-51 years). Pools of saliva and genital fluids from 10 healthy blood donors (HDs) were used as negative controls. Immunoglobulin-enriched fractions were purified from blood, genital secretions (vaginal and seminal fluids), and saliva samples and tested on a CCR5-transfected cell line. Positive binding was found in serum immunoglobulins (IgG and IgA) from 9 of 118 ESN subjects but in no HDs. All 9 sera contained anti–CCR5 IgG; notably, 8 ESN sera (except ESN no. 34) also contained anti–CCR5 IgA antibodies. One individual (ESN no. 108) only presented serum IgA and serum IgG (Table 1). CCR5 binding of all 8 IgA from positive ESNs were significantly higher than those observed with HD IgA (P < .0001).

Interestingly, all 6 subjects (5 males and 1 female) presenting anti–CCR5 IgA in genital fluids did also possess anti–CCR5 IgA in saliva.

Mucosal anti–CCR5 antibodies were further tested for binding specificity on a panel of 5 synthetic peptides spanning the extracellular loops of CCR5 and a sixth, unrelated, control peptide. Both anti–CCR5 IgG and IgA recognized a 13-mer peptide, corresponding to the second extracellular loop of CCR5 (Table 1). This 90-103 peptide contains a conformational epitope, uniquely recognized by anti–CCR5 antibodies from ESN subjects under native conditions (data not shown).

Anti–CCR5 immunoglobulins from each ESN sample and a pool of 10 CCR5-negative immunoglobulins from HDs also were tested in virus entry-neutralization assays, as previously reported.⁶ Briefly, plasmid (pCAGGS) was used to express membrane-bound envelope (env) of the primary R5 isolate JR-FL. Visceral stomatitis virus G (VSV-G) was used as a negative control virus. Pseudoviruses (kind gift of J. Binley and D. Burton) were produced by transfection of 293T cells with pNL4-3.Luc.R-E and Env-expressing pCAGGS-based plasmids. Single-round infections were performed using U87.CD4.CCR5, and luciferase activity was

<table>
<thead>
<tr>
<th>ESN no.</th>
<th>Age, y/sex</th>
<th>IgG</th>
<th>IgA</th>
<th>Genital</th>
<th>Salivary</th>
<th>Binding specificity on CCR5 peptides covering extra-membrane region of CCR5 by mucosal IgA</th>
<th>SOS pseudovirus neutralization by mucosal IgA†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>aa 14-34 aa 90-103 aa 167-178 aa 178-197 aa 260-274</td>
<td>HIV-R5 genital/ salivary VSV-G genital/ salivary</td>
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<tr>
<td>53</td>
<td>22/M</td>
<td>6780</td>
<td>6 420</td>
<td>9850</td>
<td>7100</td>
<td>–     +       –       –    –</td>
<td>2.3/0.8</td>
</tr>
<tr>
<td>55</td>
<td>38/M</td>
<td>6550</td>
<td>5 340</td>
<td>8250</td>
<td>8100</td>
<td>+     +       –       +    –</td>
<td>1.1/1.5</td>
</tr>
<tr>
<td>34</td>
<td>43/F</td>
<td>8020</td>
<td>3 000</td>
<td>250</td>
<td>250</td>
<td>–     –       +       –    +</td>
<td>&gt;40/§&lt;40/§</td>
</tr>
<tr>
<td>31</td>
<td>29/M</td>
<td>8500</td>
<td>4 800</td>
<td>6200</td>
<td>7080</td>
<td>–     +       –       –    –</td>
<td>2.8/5.8</td>
</tr>
<tr>
<td>32</td>
<td>41/M</td>
<td>6570</td>
<td>8 010</td>
<td>9650</td>
<td>8320</td>
<td>+     +       –       –    –</td>
<td>3.7/2.8</td>
</tr>
<tr>
<td>112</td>
<td>37/F</td>
<td>4650</td>
<td>5 250</td>
<td>6430</td>
<td>6660</td>
<td>–     +       –       –    –</td>
<td>0.9/1.5</td>
</tr>
<tr>
<td>108</td>
<td>32/F</td>
<td>8020</td>
<td>8 640</td>
<td>280</td>
<td>280</td>
<td>+     +       –       +    +</td>
<td>&gt;40/§&lt;40/§</td>
</tr>
<tr>
<td>109</td>
<td>35/F</td>
<td>9150</td>
<td>11 500</td>
<td>ND</td>
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<td>ND     ND       ND      ND    ND</td>
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<tr>
<td>111</td>
<td>45/M</td>
<td>6250</td>
<td>4 800</td>
<td>8010</td>
<td>6300</td>
<td>–     +       –       –    –</td>
<td>2.2/2.6</td>
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<tr>
<td>HD pool</td>
<td>Range, 25-45/5M + 5F</td>
<td>250</td>
<td>250</td>
<td>310</td>
<td>295</td>
<td>–     +       –       –    –</td>
<td>&gt;40/§&lt;40/§</td>
</tr>
</tbody>
</table>

ND indicates not determined.
*The values are expressed as counts per minute (CPM).
†The values are expressed as IC50, which correspond to 90-103–specific IgA concentration (ng/mL) yielding 50% of HIV inhibition.
‡Sequence of control peptide to VQGEESNDK.
§This value corresponds to IC50 obtained with total mucosal IgA instead of CCR5-specific IgA.
measured in the culture supernatants. Anti–CCR5 IgG and IgA, found in ESNs, efficiently neutralized HIV-R5 infectivity in vitro when gp140 env pseudotype viral strain, generated by clonal env genes (*SOS, JRFL*), was used (Table 1). Neither antibody was able to block VSV-G SOS control virus infectivity, thus confirming that their activity is specifically addressed to HIV-CCR5 strain. Similar results were obtained when HIV no. 36, an -R5 primary virus, was used to infect peripheral blood mononuclear cells (PBMCs) (Table 1).

This is the first study reporting anti–CCR5-specific IgA antibodies in mucosal secretions from ESNs. Moreover, all subjects displaying IgA in genital fluids also displayed salivary and serum IgA, therefore confirming that mucosal exposure to HIV may elicit a mucosal as well as a systemic response. Since HIV-1 is sexually transmitted, genital mucosa represents the key site for initial host-virus contact. Identification of “natural” specific immune repertoires at specific mucosal loci in ESN people could elucidate mechanisms of HIV-host interaction. The existence of natural mechanisms of mucosal protection to HIV offers an alternative noninvasive and safe way to elicit local and systemic immune protection in at-risk exposed subjects. Therefore, the study of unconventional mucosal immunity in ESN subjects should be fostered to identify potential strategies to sterile immunity or HIV infection control.

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**References**

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