CD80 expression is decreased in hyperplastic lymph nodes of HIV+ patients

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Abstract

A centrofollicular hyperplasia is present within secondary lymphoid organs during all the asymptomatic phase of the HIV disease. Although this hyperplasia has been well characterized by histological studies, the nature of the phenotypic alterations in B cell populations occurring within HIV+ lymphoid organs remains to be established. By immunohistochemistry, we thus investigated whether a particular germinal center (GC) B cell population was increased during HIV-induced hyperplasia and whether any phenotypic change was specific to HIV-1 infection. As compared to normal tonsils (three cases) and HIV– hyperplastic lymph nodes (eight patients), we observed a loss of GC polarization in all HIV+ sections (11 patients), with no more delineation between dark and light zones, as shown by Ki67, CD10, CD77, CD95 and CD86 staining. In contrast to CD86 expression which remained as intensive in HIV+ as in HIV+ lymph nodes, CD80 staining was strongly decreased in GC of HIV+ lymph nodes but not in their extrafollicular zones. The loss of CD80 expression from CD19+ B cells was also observed by cytometric analysis of cell suspensions of three HIV+ patients. Although we found no evidence of an increase in a particular GC B cell subset in HIV-1-induced hyperplasia, the strong GC disorganization observed may induce impaired cell–cell interactions and thus participate in the loss of CD80 antigen. In contrast to HIV– situations where CD80 and CD86 was similarly expressed on B cells, the lower level of CD80 expression in HIV+ GC may favor Th2 T cell responses through CD86–CD28 interactions.

Introduction

Germinal centers (GC) are the sites of antigen-driven oligoclonal B cell expansion, forming a highly specialized microenvironment essential for affinity maturation and isotype switching of antibodies and the generation of plasma and memory B cells (1). The thymus-dependent humoral response requires a network of follicular dendritic cells (FDC) and a particular population of antigen-specific GC T cells (1,2). B cell differentiation also depends on both local cytokine production and direct T–B cell interactions. B7-CD28–CTLA-4(CD152) is a ligand-receptor pair critical for effective antigen-presenting cell (APC)–T cell interactions and T cell priming. CD28–B7 interactions are also essential for B cell differentiation because CD28 and CD86 (B7.2)-deficient animals do not form GC, acquire somatic mutations and develop secondary humoral responses (3,4). CD80 and CD86 share counter-receptors, but they control Tp/2Tc/T1-type T cell responses, cytotoxic T lymphocyte generation (5), tumor growth and autoimmunity (6) differently. B7 molecules have a higher affinity for binding with CTLA-4 than with CD28, which raises the possibility that CTLA-4 is the predominant B7 receptor on activated T cells. Unlike CD28, CTLA-4 can transduce an inhibitory signal and its function may be to terminate T cell activation (6). Moreover, CD86 binding to CTLA-4 displays more rapid dissociation kinetics than CD80, and CD80 and CD86 utilize different binding determinants of the CTLA-4 molecule (7). CD28 or CTLA-4 stimulation by B7 molecules leads to the phosphorylation of multiple intracellular proteins involved in different co-stimulatory pathways (7).

CD80 and CD86 molecules also have different patterns of B cell surface expression: CD86 is expressed earlier than CD80 after B cell activation and is mostly detected on centrocytes in the light zone (LZ) of GC, whereas the strongest expression of CD80 is on centroblasts in the dark zone (DZ) (8,9). On most APC, CD86 may be constitutively expressed,
whereas CD80 is only present after activation (6). Several cytokines also modulate CD80 and CD86 expression (6,9). In addition to APC, CD80 and CD86 expression has been also detected on peripheral T cells from HIV-1-infected patients and patients with autoimmune disorders (10). Their high level of expression on T cells, probably due to continuous immune activation, is generally associated with decreased CD28 expression in patients (11,12). In vitro, repetitive stimulation of T cells also induces CD80 and CD86 expression on normal T cells (13,14). Besides their role on T cell responses, it remains to be established whether B7 molecules deliver any stimulation signal to B cells.

The HIV-1 virus is sequestered within lymphoid organs during infection, where it actively replicates, mainly in areas essential for antigen-specific T–B cell interactions. The GC microenvironment is required for HIV-1 replication (15), but GC from lymphoid organs of HIV+ patients undergo major morphological changes: prominent follicular hyperplasia during the asymptomatic phase and involution during the symptomatic phase (15). Numerous functional B cell abnormalities occur in HIV+ patients: strong polyclonal B cell activation associated with progressive loss of the antigen-specific humoral response, memory B cell and plasmocyte development dysfunction (16). No correlation has been demonstrated between these morphological and functional changes in HIV-induced hyperplasia and phenotypic changes in GC.

We used immunohistochemistry to compare the expression of a panel of antigens specific for B cells, T cells, FDC and co-stimulatory molecules. We used frozen sections of hyperplastic lymph nodes (LN) from eight HIV+ and 11 HIV-1 patients, and of normal tonsils. There was a loss of compartmentalization in HIV+ patients, with centroblasts and centrocytes randomly distributed throughout the GC and the

### Table 1. Primary mAb used

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Clone (Isotype)</th>
<th>Source (ref.)</th>
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<tbody>
<tr>
<td>CD4</td>
<td>Q4120</td>
<td>Q. Sattentau (35)</td>
</tr>
<tr>
<td>CD8</td>
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<td>BL13</td>
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<tr>
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<td>To15 (IgG2b)</td>
<td>Dako</td>
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<td>MHIM6</td>
<td>Dako</td>
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<tr>
<td>CD40</td>
<td>G28.5</td>
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<tr>
<td>CD57</td>
<td>NK-1 (gM)</td>
<td>Novocastra</td>
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<tr>
<td>CD77</td>
<td>38.13 α-Gb3 (gM)</td>
<td>J. Wils (36)</td>
</tr>
<tr>
<td>CD80</td>
<td>BB1 (gM)</td>
<td>Serotech</td>
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<tr>
<td>CD80</td>
<td>L307.4</td>
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</tr>
<tr>
<td>CD86</td>
<td>B-T7</td>
<td>Biotest</td>
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<td>CD95</td>
<td>UB2</td>
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<tr>
<td>FDC</td>
<td>12B1 (IgG2a)</td>
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<td>IgD</td>
<td>JA 11</td>
<td>Immunotech</td>
</tr>
<tr>
<td>Ki-67</td>
<td>Ki-67</td>
<td>Dako</td>
</tr>
</tbody>
</table>

All mAb were of IgG1 isotype unless otherwise indicated. Isotypic controls were purchased from ICN (Costa Mesa, CA) and Dako (IgG2b).
overnight, fixed in acetone and labeled. Tissue sections were frozen. Then 5
All specimens were collected rapidly after surgery and snap-

Immunohistochemistry

histopathology showed that all patients had follicular hyper-
respectively) were included. Informed consent was obtained

Fig. 3. CD80 expression on CD19+ B cells. Cell suspensions from

CD80 and CD86 expression

after chronic tonsillitis, were used as controls. Therapy. Tonsils removed from three seronegative patients,

19 had received antiretroviral therapy [zidovudine (AZT)
clinical stage A (patients FH 9–16) or B (patients FH 17–19)

FDC network disorganized. CD80 expression was low in the
HIV+ GC, whereas its expression was unchanged in the
extrafollicular zone, as was that of CD86. CD80 is a late B cell

Methods

Patients and biopsies

LN, taken for diagnosis, from eight seronegative patients
and 11 seropositive patients (median age 32 and 39 years
respectively) were included. Informed consent was obtained
from all patients included in this study. LN were removed from
cervical, submandibulary or axillary regions. Conventional
histopathology showed that all patients had follicular hyper-
plasia (FH) as defined by Biberfeld et al. (17). LN from
seronegative patients (FH 1–8) showed no clinical or sero-
logical evidence of immune disease. Seropositive individuals
had CD4 counts ranging from 100 to 368 x 10^6/l and were at
clinical stage A (patients FH 9–16) or B (patients FH 17–19)
according to CDC classification (18). Patients FH 16, 17 and
19 had received an antiretroviral therapy [zidovudine (AZT)
+ lamivudine (3TC) or didanosine (ddi)] achieved 3 or 6
months before biopsy. Otherwise patients had received no
therapy. Tonsils removed from three seronegative patients,
after chronic tonsillitis, were used as controls.

Immunohistochemistry

All specimens were collected rapidly after surgery and snap-
frozen. Then 5 µm thick cryostat sections were cut, air-dried
overnight, fixed in acetone and labeled. Tissue sections were
stained with the universal LSAB kit (horseradish peroxidase)
from Dako Patts (Glostrup, Denmark). The primary mAb used
are listed in Table 1.

Cytometric analysis of CD80 and CD86 expression

After gentle dilaceration with forceps, cell suspensions from
tonsils and lymph nodes were incubated for 1 h at 4°C in
PBS containing 1% BSA (Fraction V, Sigma), 0.1% NaN3
(staining buffer) and 10% normal human heat-inactivated AB+
s serum to avoid non-specific binding. CD80 and CD86 antigen
expression on B cells was detected by phycoerythrin (PE)-
conjugated L307.4 (IgG1; Becton Dickinson, San Jose, CA)
and FITC-conjugated B-T7 (IgG1; Diacalone, Besançon,
France) mAb respectively. After two washes (5 min, 700 g,
4°C), cells were stained with a FITC- or PE-conjugated CD19
mAb (BC-3, IgG1; Diacalone). After extensive washes, cells
were fixed in PBS containing 1% paraformaldehyde. Immuno-

Results and discussion

Unlike normal antigen challenge in which hyperplasia is
transitory, FH is persistent and extensive throughout the
asymptomatic phase of HIV infection. It is probably maintained
by continuous replication of HIV-1 in lymphoid organs. Numer-
ous studies have shown that B cell function is highly impaired
by HIV infection (16,19). Lymphoid organs are the principal
sites of B cell differentiation (1), so we compared B cell
populations in HIV+ and HIV− hyperplastic lymphoid organs.

We observed enlarged, irregularly shaped GC surrounded
by a limited, frequently disrupted, IgD+ mantle zone (MZ) in
hyperplastic HIV+ LN, as previously shown (20–22) (Fig. 1F).
CD4+ T cells were scattered in the GC of HIV+ patients rather
than being limited to the LZ as in normal tonsils and HIV− LN.
There was a significant increase in CD57+ cells in the
extrafollicular zone and in CD8+ T cells in the GC of hyperplas-

CD80 CD86

Normal Tonsil

A

B

Patient FH9

C

D

Fluorescence Intensity (Log)

Fig. 3. CD80 expression on CD19+ B cells. Cell suspensions from
one tonsil and one HIV+ patient (FH9) were labeled with PE-CD80
and FITC-CD19 mAb (A and C) or with FITC-CD86 and PE-CD19
mAb (B and D). Analysis was performed on 10,000 viable cells. Open
and filled histograms are the control and CD80 or CD86 labeling
respectively.

CD80 expression on CD19+ B cells was decreased in HIV+
patients rather

than being limited to the LZ as in normal tonsils and HIV− LN.
We also observed FDC network disorganization in HIV+ patients,
but CD21, CD22 and CD40, but polarization of the HIV+ GC was
lost, as shown by Ki67, CD10 and CD77 staining, showing
random distribution of centroblasts throughout the GC (Fig. 1E). There is no obvious marker for centrocytes but they express no Ki67 and CD77, remain CD38+ and CD95+, and increase their expression of CD86 (30). Thus, we observed that centrocytes, present only in the LZ of normal and hyperplastic HIV+ GC, were distributed throughout the GC of HIV+ patients (Fig. 2F). It is therefore unlikely that a particular population of GC B cells increases during HIV-induced hyperplasia.

CD86+ cells limited to the LZ of normal tonsils and HIV+ hyperplastic LN, were dispersed throughout the GC in HIV+ patients (Fig. 2F), whereas the intensity of CD86 staining was similar for all studied sections. In tonsil sections, CD80 staining overlaps the dark and light zone with a small ring of unstained cells limited to the LZ (IgD+) and CD86+ areas (Fig. 2A). In a few cases, CD80 expression was higher in the LZ of HIV+ LN than in the DZ. This result differs with that of Vyth-Dreese et al., suggesting that CD80 is expressed mainly in the DZ of GC (8). This may be due to the use of different CD80 mAb, BB1 or L307.4 versus B7.24 mAb. We obtained a similar pattern of staining with CD80 and CD23 mAb, suggesting that CD80 is expressed by both GC B cells and FDC. In HIV+ LN, CD80 staining was weak (six patients, FH 9–13 and 16) or absent (five patients, FH 14, 15 and 17–19), whereas CD80 staining was still observed in all HIV+ hyperplastic GC. We did detect CD86 and CD80 expression on extrafollicular dendritic cells from most sections.

As CD80 and CD86 molecules are expressed by most APC, including FDC and DC, present in lymphoid organs, we also studied the expression of these two markers on isolated cell suspensions from normal tonsils and three HIV+ patients (FH 9, 17 and 19). Cell suspensions were thus double labeled with CD19 and either CD80 or CD86 mAb. As shown in Fig. 3, CD80 and CD86 expression was similar on CD19+ B cells from tonsil, whereas CD80 expression was lost from the surface of CD19+ B cells of patient FH 9. Similar results were obtained for the two other HIV+ patients tested (FH 17 and 19). In contrast, CD86 was similarly expressed in these three patients and in the control. Due to the paucity of DC and FDC in cell suspensions, it was impossible to be precise whether CD80 and CD86 expression was changed on these cells. There was no correlation between the magnitude of the decrease in CD80, the stage of the disease, the antiretroviral therapy and the number of circulating CD4+ T cells. In contrast to previous data showing that peripheral HIV+ T cells express CD80 and CD86 antigens (14), we did not detect them on T cells from HIV+ LN sections.

Studies in vitro have shown that CD80 is a late activation marker of APC whose expression requires CD86–CD28 and CD40–CD40L interaction (9,31), suggesting that the low level of CD80 expression in HIV+ patients results from inadequate T–B or FDC–B interactions and/or is associated with GC depolarization. Soluble factors released by T cells may also affect expression of CD80 on B cells (6,9). The loss of CD80 may lead to more CD86–CD28 interactions, increasing HIV-1 replication within the GC (32) and leading to a shift towards Tc2 T cell responses, as previously reported by Klein et al (33). It has been suggested that preferential interactions occur between CD80 and CTLA-4 antigens (9). CTLA-4 may exert a feedback control on T cell response (34), so the loss of CD80 may be involved in maintaining hyperplasia.

Thus, hyperplasia affects both centroblast and centrocyte populations in HIV+ LN. Despite the strong GC depolarization typical of HIV+ hyperplasia, the expression of most B cell markers was similar in HIV+ and HIV+ lymph nodes. The most striking result is the loss of CD80 from the GC, with CD86 levels unchanged. It will be interesting to test whether tritherapy restores expression of CD80 molecules in lymphoid organs from HIV patients. In animal models, it would be interesting to investigate whether a CD80 decrease appears as early as the acute phase of the disease.

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Abbreviations

APC antigen-presenting cell
DZ dark zone
FDC follicular dendritic cells
FH follicular hyperplasia
GC germinal center
LN lymph node
LZ light zone
MZ mantle zone
PE phycoerythrin

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