Alterations of self-reactive antibody repertoires in HIV disease: An insight into the role of T cells in the selection of autoreactive B cells

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Abstract

Infection with human immunodeficiency virus (HIV) is characterized by a progressive depletion of CD4+ T cells that parallels a dysfunction of the B cell compartment and a disturbed recognition of self-antigens. The relationship between T lymphocyte homeostasis and abnormalities in the selection of self-reactive B cells is not clear as yet. We have therefore compared repertoires of natural antibodies of healthy donors and of patients at various stages of HIV infection. The reactivity of IgM and IgG antibodies in plasma of healthy blood donors and of HIV-positive patients with high and low CD4+ T cell counts was assessed by semi-quantitative immunoblotting using self-antigens extracted from normal human tissues. Repertoires of reactivities were compared between groups of individuals by means of multivariate parametric statistical analysis. We observed that repertoires of self-reactive IgM and IgG from HIV-seropositive patients exhibited significantly altered patterns of reactivity, as compared to those of healthy controls. Further, self-reactive repertoires of IgM and IgG of patients with high CD4+ T cell counts differed significantly from those of patients with low CD4+ T cell counts. A longitudinal analysis of self-reactive antibody repertoires of progressor and non-progressor patients suggested an influence of CD4+ T cell counts on immunoglobulin reactivity toward self-antigens. These observations support the hypothesis that altered T cell/B cell interactions due to altered CD4+ T cell help severely impact on the selection of self-reactive antibody repertoires and may contribute to the onset of pathological autoimmune in HIV disease.

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1. Introduction

Defective immune homeostasis in HIV disease is characterized by a progressive decrease in the number and function of CD4+ T cells. Alterations of the B cell compartment include polyclonal B cell activation and high levels of self-reactive antibodies in serum. Natural antibodies of the IgM, IgG, and IgA isotype that are reactive with a broad range of self-antigens are known to be present in normal human serum. A role for T cells in the selection of self-reactive antibody repertoires has been documented in the mouse, but so far not in humans.

Analysis of autoantibodies at the level of investigation of single components of the biological system, such as isolated autoantibodies and purified autoantigens or recombinant peptides, proved not to be suitable to clarify the relationship between T lymphocyte homeostasis and abnormalities in the selection of self-reactive B cells. Taking HIV disease as a model, we have therefore compared repertoires of natural antibodies of healthy donors and of patients at various
Stahl
CD4
donors
in
from
with
fiv
quantitative
reaction
complex
antibody
mixtures
plasma
toward
compact
antigen
mixtures
extracted
human
cells
tissues.
Experimental
design
changes
complex
interactions
dependence
variables
such
time,
principally
us
depict
functional
dynamics
investigated
system.
Detailed
analyzed
IgM
and
sero-reactive
antibody
repertoires
HIV-seropositive
patients
high
and
low
CD4+ T
cell
counts.
Study
longitudinal
approach
analyzing
antibody
repertoires
samples
patients
fast/
slow
progression
HIV
observation
time
ranging
months.
Demonstrate
self-reactive
antibody
repertoires
sero-reactive
patients.
Results
notion
selection
self-reactive
antibody
repertoires
humans
T
cell
dependent.

2. Materials and methods

2.1. Sources of antibodies

Heparin-plasma
samples
obtained
20
HIV-
seropositive
patients
males
6
females
CD4+
T
cell
counts
1000
19
HIV-seropositive
patients
2
women
CD4+ T
cell
counts
50
11
HIV-seronegative
blood
(16
males
4
females).
Viremia
16
patients
CD4+ T
cell
counts
1000
15
patients
CD4+ T
cell
counts
50
1.2
HIV
RNA/ml
(mean±S.D.;
range
113.334
327)
patients
CD4+ T
cell
counts
50
Differences
viral
load
statistical
significance
Mann–Whitney
U-test).
Frozen
plasma
obtained
10
patients
SEROCO
HIV-seropositive
individuals
supported
Agence
Deutches
le
SIDA
(AANRS),
France;
samples
different
time
points
each
progressor
non-progressor
patients.
Progressor
patients
defined
remaining
700
10
years
CD4+ T
cell
counts
50
within
3
years
follow-up;
non-progressor
patients
defined
CD4+ T
cell
counts
1000
within
years
cohort.
CD4+ T
cell
counts
non-progressor
progressor
patients
1061 ± 446
11
mean±S.D.;
898 ± 239
11,
samples
collected
point
1,
861 ± 168
212 ± 69
11,
samples
collected
point
5.
CD4+ T
cell
counts
between
progressor
patients
statistically
significant
point
5
0.009,
Mann–Whitney
U-test),
not
point
1
0.6015.
Patients
did
not
anti-viral
therapy,
sera
collected
heterologous
anti-viral
therapy
available.

Immunoglobulin
concentrations
determined
nephelometry.
Concentration
IgM
IgG
patients
CD4+ T
cell
counts
1000
1.47 ± 0.86
g/l
(mean±S.D.;
ranging
7.00
3.60
g/l)
12.90 ± 3.03
g/l
(6.63–32.00
g/l),
respectively.
Concentration
IgM
IgG
HIV-positive
patients
CD4+ T
cell
counts
1000
1.31 ± 0.76
g/l
(0.37–3.42
g/l)
15.80 ± 8.50
g/l
(8.29–43.00
g/l).
The
concentration
IgM
IgG
healthy
blood
donors
1.22 ± 0.69
g/l
(0.39–3.11
g/l)
9.46 ± 1.88
g/l
(6.16–12.70
g/l),
respectively.
Samples
progressor
patients
SEROCO
study,
plasma
concentration
IgM
IgG
1.79 ± 0.67
g/l
(0.9–2.57
g/l)
15.05 ± 4.50
g/l
(7.32–23.90
g/l),
respectively.
Samples
non-progressor
patients,
plasma
concentration
IgM
IgG
1.57 ± 0.86
g/l
(0.9–2.57
g/l)
17.79 ± 5.42
g/l
(11.10–33.00
g/l).
Differences
plasma
concentration
IgM
statistical
significance
comparison
patients
CD4+ T
cell
counts
1000
patients
CD4+ T
cell
counts
1000
healthy
individuals
0.417)
patients
CD4+ T
cell
counts
50
healthy
individuals
0.002),
not
comparison
patients
CD4+ T
cell
counts
1000
patients
CD4+ T
cell
counts
50
patients
non-progressor
progressor
patients
point
1
0.9168,
non-progressor
progressor
patients
point
5
0.2506.
IgG
purified
affinity
chromatography
protein
G-Sepharose
Pharmacia
Biotech,
Uppsala,
Sweden.
IgG
then
ELISA
SDS-PAGE.
Normal
human
IgG
therapeutic
use
intravenous
immunoglobulin,
(IgG),
Sangoglobulin®,
a
Central
Laboratory
Swiss
Red
Cross
(Bern,
Switzerland),
reference
normal
IgG.
Reference
preparation
normal
IgM
(IgM)obtained
submitting
Pentaglobulin®
(Hitoxin
Pharma,
Dreieich,
Germany),
Enriched
therapeutic
preparation
normal
human
immunoglobulin,
size
exclusion
chromatography
Sephacryl
HR
S-300.
2.2. Analysis of antibody repertoires by quantitative immunoblotting

For analysis of antibody repertoires, we used a quantitative immunoblotting technique that allows for the simultaneous assessment of antibody reactivities of different sources with large numbers of antigens in normal homologous tissue extracts of human liver, kidney, and stomach obtained during surgical procedures. These extracts were chosen because of their richness in different antigens, thereby offering a large panel of antigens and allowing for a reliable distinction between repertoires of different groups of subjects. Soluble extracts were prepared from tissues in 2% SDS, 1.45 M 2-mercaptoethanol, 125 mM Tris–HCl, pH 6.8, containing 1.0 μg/ml aprotinin, 1.0 μg/ml pepstatin A, and 1.0 mM EDTA on ice. The samples were sonicated four times for 30 s to disrupt DNA before being centrifuged, boiled for 5 min and dialyzed against PBS, pH 7.4. Solubilized proteins were subjected to preparative SDS-PAGE in 10% polyacrylamide. The amount of proteins subjected to electrophoresis ranged between 400 and 600 μg/ml, depending on the tissue extract, and was maintained constant for a given tissue in all experiments. The proteins were transferred onto nitrocellulose (Schleicher & Schuell, Dassel, Germany) for 1 h at 0.8 mA/cm² using a semi-dry electroblotter model A (Ancos, Denmark). Membranes were blocked with PBS containing 0.2% Tween 20 for 1 h at room temperature. The antibodies to be tested were then incubated for four hours at room temperature with the membranes following the addition of one sample per slot in a cassette miniblot system (Immunetics Inc., Cambridge, MA). The membranes were washed and revealed with µ-chain-specific secondary rabbit anti-human IgM antibody or γ-chain-specific secondary rabbit anti-human IgG antibody coupled to alkaline phosphatase (Dako, Glostrup, Denmark) for 90 min at room temperature. Immunoreactivities were revealed using the NBT/BCIP (nitroblue tetrazolium/S-bromo-4-chloro-3-indolyl-phosphate) substrate (Sigma, St. Louis, MO). IgM was tested at a concentration of 20 μg/ml, and IgG at a concentration of 200 μg/ml. The V-region dependency of recognition of blotted antigens by the immunoglobulins tested has been documented previously. Densitometric quantification of immunoreactivities was performed by scanning the membranes (SnapScan 600, Agfa Gevaert, NY). Blotted proteins were then stained with colloidal gold (Protogold, BioCell, Cardiff, GB) and subjected to a second densitometric analysis to record the protein profile and to quantitate transferred proteins. Data were analyzed using a Power Mac G3 computer (Apple Computer Inc., Cupertino, CA) and the IGOR software (Wavemetrics, Lake Oswego, OR). Densitometric profiles of immunoreactivity were compared by referring to their corresponding protein profile following correction of the migration defects by superimposition of protein peaks. A sample of the reference IgM (IgM) or IgG (IgG) was included in each blot allowing to rescale different membranes transferred with a given protein extract and to adjust for the intensity of staining of different membranes. Migration distances (X-axis) and optical density (Y-axis) were expressed as arbitrary units (a.u.). Migration distances of 200, 600 and 1000 a.u. corresponded to apparent molecular weights of 200, 65, and 20 kDa, respectively. The assay is reproducible with a 10% variation coefficient. The 95% confidence interval of the mean area under the curve corresponding to each peak of immunoreactivity is 30% in the case of IgM, and 25% in the case of IgG, as calculated using Student’s t-test.

2.3. Statistical analysis

Multivariate statistical treatment of the data was performed by means of the IGOR software, including specifically written software packages and the StatView software. Densitometric profiles of reactivity of IgM and IgG of patients and healthy controls with antigens in each tissue extract were divided into sections corresponding to individual peaks of immunoreactivity. Peak areas under the curve were calculated for each section. In order to discriminate between groups of individuals, areas corresponding to sections were submitted to principal component analysis (PCA). The PCA discriminates the repertoires of reactivities of antibodies from different individuals and summarizes, with minimum loss of information, the multidimensional information that is accounted for by the values of peak areas in all the sections, into a two-dimensional graph (factor 1 versus factor 2). In the present study, the amount of information taken into account by the PCA varies between 50 and 85%, depending on the tissue extracts. These percentages represent the eigenvalues that characterize each of the factors generated by the PCA. Each symbol in a PCA graph represents the repertoire of antibody reactivities of a single individual. The significance of the discrimination between antibody repertoires of groups of individuals was investigated by submitting the PCA data to linear discriminant analysis (LDA), and by subsequently comparing factors 1 of the LDA by means of a Mann–Whitney U-test. Differences were considered to be significant, if p values were <0.05 as assessed by the Mann–Whitney U-test. The PCA of repertoires of antibody reactivities performed individually for each group of individuals allowed the calculation of respective variances. Variances were compared using the F-test. For more detailed background information on the multivariate analysis of blot data, we refer to recent literature from colleagues and from our group.

For analysis of longitudinal data, a mixed linear model as fitted to the first factor obtained by PCA upon analysis of IgG repertoires of progressor and non-progressor patients at time point 1 and time point 5, using the PROC MIXED procedure of the SAS® system software (SAS Institute Inc., Cary, NC). The impact of the CD4⁺ T cell count, of the progressor versus non-progressor status of the patients and the impact of this status on changes over time were tested. Within this model, p <0.05 corresponds to a significant impact on the expression of self-reactive antibody repertoires, and a p <0.1 to a tendency.
3. Results

3.1. Comparative analysis of self-reactive antibody repertoires of healthy individuals and HIV-seropositive patients

3.1.1. Antibody repertoires of IgM
Self-reactive antibody repertoires of IgM of patients with high CD4+ T cell counts and healthy controls were discriminated by PCA (p < 0.002 in the case of all tissue extracts tested). Multiparametric statistical analysis also discriminated between reactivities of IgM of patients with low CD4+ T cell counts and those of healthy controls (p < 0.011 in the case of all tissue extracts tested). The calculation of the individual variances of repertoires of IgM of HIV-seropositive patients with high CD4+ T cell counts and with low CD4+ T cell counts and of healthy individuals toward self-antigens indicated that heterogeneity of groups of individuals might contribute to differences between antibody repertoires in the case of immunoreactivities of IgM toward liver extracts (data not shown).

3.1.2. Antibody repertoires of IgG
Self-reactive antibody repertoires of IgG of patients with high CD4+ T cell counts and controls were discriminated by PCA (p < 0.006 in the case of all tissue extracts tested), as well as those of patients with low CD4+ T cell counts and healthy controls (p < 0.001 in the case of all tissue extracts tested). The calculation of the individual variances of repertoires of self-reactive IgG indicated that heterogeneity of groups of individuals might contribute to differences between antibody repertoires of HIV-seropositive patients with high CD4+ T cell counts and with low CD4+ T cell counts and of healthy individuals in the case of immunoreactivities of IgG toward stomach extracts (data not shown) and to differences between antibody repertoires of HIV-seropositive patients with high CD4+ T cell counts and of healthy individuals in the case of immunoreactivities of IgG toward kidney extracts (data not shown).

In all of the experiments analysing self-reactive IgM and self-reactive IgG, there were no single antigenic bands recognized differentially by autoantibody repertoires of groups of individuals that would account for differences as revealed by multiparametric statistical analysis (data not shown).

3.2. Comparative analysis of self-reactive antibody repertoires of HIV-seropositive patients with high and low CD4+ T cell counts

Self-reactive antibody repertoires of IgM of patients with high CD4+ T cell counts and patients with low CD4+ T cell counts were discriminated by PCA (p < 0.042, in the case of all tissue extracts tested). PCA also discriminated between antibody repertoires of IgG of patients with high CD4+ T cell counts and patients with low CD4+ T cell counts (p < 0.01), (Fig. 2). The calculation of the individual variances of repertoires of IgM and of IgG of patients indicated homogeneity of groups of individuals (Fig. 1). Patterns of reactivity of IgM and IgG of patients with high and with low CD4+ T cell counts were homogeneous among patients in the same groups; we did not observe differences in the nature of the antigenic bands recognized by the antibodies of either group of patients that would account for differences as revealed by multiparametric statistical analysis (data not shown).

When PCA was performed as a cumulative PCA on all of the tissue extracts tested, self-reactive antibody repertoires of IgM of patients with high CD4+ T cell counts and patients with low CD4+ T cell counts were discriminated at a level of p = 0.0012, and self-reactive antibody repertoires of IgG of patients with high CD4+ T cell counts and patients with low CD4+ T cell counts were discriminated at a level of p = 0.0002. Groups of patients were then divided at the basis of plasma viremia, and not at the basis of CD4+ T cell counts. A threshold for the viral load of 500 copies HIV RNA/ml was applied, since it was the median of the viral load for all of the patients, including progressors and non-progressors. The cumulative PCA performed on all of the tissue extracts tested discriminated self-reactive antibody repertoires of IgM of patients exhibiting a viral load <500 copies HIV RNA/ml and of patients exhibiting a viral load >500 copies HIV RNA/ml at a level of p = 0.0073, and self-reactive antibody repertoires of IgG of patients exhibiting a viral load >500 copies HIV RNA/ml and of patients exhibiting a viral load <500 copies HIV RNA/ml at a level of p = 0.001. When subdividing the group of patients with high CD4+ T cell counts in two subgroups based on the viral

<table>
<thead>
<tr>
<th>IgM</th>
<th>Liver 2.53*</th>
<th>2.37*</th>
<th>1.06</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kidney 1.62</td>
<td>1.33</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>Stomach 1.22</td>
<td>1.32</td>
<td>1.08</td>
</tr>
<tr>
<td>IgG</td>
<td>Liver 1.17</td>
<td>1.20</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>Kidney 2.17*</td>
<td>1.85</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>Stomach 3.18*</td>
<td>2.56</td>
<td>1.28</td>
</tr>
</tbody>
</table>

Total variances of reactivities of IgM in whole plasma and of IgM purified from plasma with self-antigens in extracts of liver, kidney, and stomach were calculated separately in a 33-dimensional vector space, depending on the source of immunopeptides and the tissue extract. Significance of differences between total variances were assessed by the F-test. Significant differences indicate a heterogeneity of the repertoires of IgM reactivities between two groups of individuals, and are indicated by asterisk (*).
Fig. 1. Comparative analysis of the patterns of reactivity of IgM of healthy individuals and HIV-positive patients with self-antigens. IgM in plasma of HIV-positive patients with high and low CD4+ T cell counts and of healthy blood donors was immunoblotted at 20 μg/ml with antigens in extracts of self-antigens, as described in Section 2 for each individual, the densitometric profile of reactivity with a given tissue extract was divided into sections corresponding to peaks of reactivity. Respective peak areas were calculated in the case of each tissue extract. The data were subjected to PCA within a 45–49-dimension vector space, depending on the tissue extract, and fitted within a two-dimensional linear subspace (factor 1/factor 2). PCA discriminated between repertoires of patients and controls in the case of all tissue extracts tested (0.0001 < p < 0.026, depending on the tissue extract, by the Mann–Whitney U-test). Depicted are the results of the comparative analysis of patterns of reactivity toward liver, kidney and stomach antigens between healthy individuals and HIV-positive patients with high CD4+ T cell counts (panel A) and between healthy individuals and HIV-positive patients with low CD4+ T cell counts (panel B). Percentages of variance accounted for by factor 1 and factor 2 are indicated on the abscissa and ordinate, respectively. Each symbol represents the reactivity of IgM of a single individual for the group of HIV patients (filled circle) and healthy blood donors (open square).

load, the cumulative PCA did however not discriminate between patients with high CD4+ T cell counts and a viral load >500 copies HIV RNA/ml and patients with high CD4+ T cell counts and a viral load <500 copies HIV RNA/ml (p = 0.3051 in the case of immunoreactivities of IgM; p = 0.1356 in the case of immunoreactivities of IgG).

3.3. Self-reactive antibody repertoires of progressor and non-progressor patients

The patterns of reactivity of IgM and IgG of patients with a rapidly progressive disease and with a slowly progressing disease were similar: we did not observe differences
between progressors and non-progressors in the nature of the antigenic bands recognized by IgM and IgG

A multivariate analysis of the longitudinal data was performed using a mixed linear model as explained in Section 2. The model suggested an impact of the CD4+ T cell counts on the expression of IgM and IgG antibody repertoires toward kidney antigens (p < 0.075 and p < 0.061, respectively), but no impact of CD4+ T cell counts on the expression of Ig
repertoires toward liver and stomach antigens. The pro-
gressor status of the patients was associated with an altered
expression of the IgM repertoire towards stomach antigens
\( (p = 0.007) \) and of the IgG repertoire towards liver antigens
\( (p = 0.067) \). The change over time of the expression of
the IgG repertoire towards stomach antigens was dif-
erent between progressor and non-progressor patients
\( (p = 0.079) \).

### 4. Discussion

Polyclonal B cell activation and high titers of autoantibod-
ies reactive with e.g. HLA molecules, CD95, phospholipids,
human \( \beta 2 \) glycoprotein-1, neutrophil cytoplasmic antigens,
are frequently observed in untreated HIV-infected individuals
\[24\–26\]. Disturbances in mechanisms of self-recognition were
shown to parallel the progression of HIV-1 infection \[27\–28\].
The relationship between acquired B cell abnormalities in
HIV infection and defective T cell homeostasis, however, re-
 mains unclear.

Under physiological conditions, self-reactive B cells are
positively selected \[29\] and autoantibodies of the IgG,
IgM and IgA isotypes are present in normal serum \[30\].
Few data document a role for T cells in the selection of self-
reactive antibody repertoires. The production of self-reactive
immunoglobulins by \( \text{CD}^{+} \)-B-1a cells \[31\] in infected mice is con-
trolled by T cells \[32\].
The total pool of CD5\(^{+}\) B cells is smaller in athymic Balb/c mice than in euthymic normal mice
\[10\].
The absence of T lymphocytes in nude mice was
shown to correlate with a decreased frequency in precursors
of anti-erythrocyte autoreactive B cells and with perturbed
self-reactive antibody repertoires of IgM as compared with
normal euthymic mice \[33\].
The analysis of MHC-congenic mouse strains demonstrated an MHC-dependent control of nat-
ural antibody repertoires that is likely to operate through dif-
ferential selection of T cell repertoires \[34\].

There is a large body of evidence that pathological B

cell mediated autoimmunity and altered T cell functions are
related. Patients with mycosis fungoides, a T cell lineage
lymphoma, show a significant increase in incidence of
lymphocytotoxic antibodies \[35\].

Patients with angioim-

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**Fig. 3.** Comparative analysis of the patterns of reactivity of IgM of HIV-positive patients with high and with low CD4\(^{+}\) T cell counts. The data of peak areas calculated for the reactivity of IgM of HIV-positive patients with high and with low CD4\(^{+}\) T cell counts, obtained as explained in the legend of Fig. 2, were subjected to PCA within a 33–49-dimension vector space, depending on the tissue extract, and fitted within a two-dimensional linear subspace (factor 1/factor 2). PCA discriminated between repertoires of patients with high and with low CD4\(^{+}\) T cell counts in the case of all tissue extracts tested \( (0.003 < p < 0.043) \), depending on the tissue extract, by the Mann-Whitney \( U \)-test. Percentages of variance accounted for by factor 1 and factor 2 are indicated on the abscissa and ordinate, respectively. Each symbol represents the reactivity of antibody of a single individual for the group of HIV patients with high CD4\(^{+}\) T cell counts (filled circle) and for the group of HIV patients with low CD4\(^{+}\) T cell counts (open square).
munoblastic lymphadenopathy and dysproteinemia (AILD) often have evidence of autoimmune disease, that may be related to a deficiency in suppressor T cell function resulting in proliferation of self-reactive B cells with autoantibody production and polyclonal gammapathy. T-cell activation, used in controlling B cell-mediated autoimmune diseases, further emphasizing the influence of T cells on the selection of self-reactive antibody repertoires under pathological conditions. The study of HIV disease may provide an insight into the relationship between T lymphocytes and the selection of self-reactive B cell repertoires in humans. The present study demonstrates a significant bias in repertoires of reactivities of IgM and IgG of HIV-seropositive patients toward self-antigens as compared with reactivities of IgM and IgG in the serum of healthy individuals. In addition, multiparametric statistical analysis discriminated the patterns of reactivity of self-reactive IgM and IgG between HIV-positive patients with high and low CD4+ T cell counts. Further subdividing each group of patients according to the viral load did not indicate a role of plasma viremia for the discrimination of self-reactive antibody repertoires of patients with high CD4+ T cell counts and patients with low CD4+ T cell counts, as indicated by the finding that antibody repertoires of IgM and of IgG were similar between the two groups of patients. Our data thus suggest that, although a functional disturbance of B and T cell interactions within the overall B and T cell repertoire might critically depend on plasma viremia, the interaction of self-reactive B and T cells is more critically influenced by CD4+ T cell counts than by plasma viremia. The analysis of antibody repertoires of individual patients followed up longitudinally indicated that the expression of IgM and IgG antibody repertoires toward some self-antigens is dependent on the number of CD4+ T cells. Our observations are consistent with previous findings of defective self-reactive antibody repertoires in patients with the hyper-IgM syndrome and with altered T-B cell interactions. Interestingly, a decreased induction of CD40L in CD4+ T cells in HIV patients with low CD4+ T cells counts has been reported. The latter may contribute to altered T-B cell interactions in HIV disease that explain an altered development of natural self-reactive antibody repertoires in T cell deficient humans.

Self-reactive B cell repertoires were altered both in patients with severely decreased CD4+ T cell counts and in

![Fig. 4. Comparative analysis of the patterns of reactivity of IgG of HIV-positive patients with high and with low CD4+ T cell counts. The data of peak areas calculated for the reactivity of IgG of HIV-positive patients with high and with low CD4+ T cell counts, obtained as explained in the legend of Fig. 2. PCA discriminated between repertoires of patients with high and with low CD4+ T cell counts in the case of all tissue extracts tested (0.0003 < p<0.019, depending on the tissue extract, by the Mann-Whitney U-test). Percentages of variance accounted for by factor 1 and factor 2 are indicated on the abscissa and ordinate, respectively. Each symbol represents the reactivity of antibody of a single individual for the group of HIV patients with high CD4+ T cell counts (filled circle) and for the group of HIV patients with low CD4+ T cell counts (open square).](image-url)
patients whose CD4⁺ T cells were relatively conserved, suggesting that the count of CD4⁺ T cells might not be the only factor influencing selection of self-reactive B cells throughout all of the highly differentiated stages of development of HIV disease. The early stage of HIV infection is characterized by normal total CD4⁺ T cell counts and by a non-perturbed distribution of TCR-BV CDR3 populations of CD4⁺ T cells, both in blood and in lymphoid tissues. The chronic immune activation in early HIV disease however translates into an increase in cytokine production that may influence the selection of self-reactive B cell repertoires in early disease stages via T cell independent mechanisms. Changes in autoreactive B cell repertoires due to T cell independent mechanisms might thus precede changes in autoreactive B cell repertoires due to T cell homeostasis. The observation that self-reactive B cell repertoires were altered both in patients with severely decreased CD4⁺ T cell counts and in patients whose CD4⁺ T cells were relatively conserved could also result from alterations in a subpopulation of regulatory CD4⁺ T cells that are not accounted for by the enumeration of total CD4⁺ T cells. In summary, we consider altered self-reactive antibody repertoires in HIV disease to be a read-out of perturbations in the natural physiological repertoire of immunoglobulin self-reactivities that accompany progression of HIV infection in relation to alterations of the total CD4⁺ T cell count.

Taken together, repertoires of reactivities of IgM and IgG of HIV-seropositive patients toward self-antigens differ from those in the serum of healthy individuals, and patterns of reactivity of self-reactive IgM and IgG of HIV-positive patients with high and with low CD4⁺ T cell counts are clearly discriminated by multiparametric statistical analysis. These observations support the concept that T cells contribute to the selection of natural self-reactive antibody repertoires in humans. It is as yet unknown whether restoration of CD4⁺ T cell homeostasis in patients treated with highly active anti-retroviral therapy is associated with a reinstatement of a normal pattern of self-recognition of B cells.

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