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Decoupling activation and exhaustion of B cells in spontaneous controllers of HIV infection

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Abstract

Objective—To define the impact of chronic viremia and associated immune activation on B-cell exhaustion in HIV infection.

Design—Progressive HIV infection is marked by B-cell anergy and exhaustion coupled with dramatic hypergammaglobulinemia. Although both upregulation of CD95 and loss of CD21 have been used as markers of infection-associated B-cell dysfunction, little is known regarding the specific profiles of dysfunctional B cells and whether persistent viral replication and its associated immune activation play a central role in driving B-cell dysfunction.

Methods—Multiparameter flow cytometry was used to define the profile of dysfunctional B cells. The changes in the expression of CD21 and CD95 were tracked on B-cell subpopulations in patients with differential control of viral replication.

Results—Although the emergence of exhausted, CD21^{low} tissue-like memory B cells followed similar patterns in both progressors and controllers, the frequency of CD21^{low} activated memory B cells was lower in spontaneous controllers.

Conclusion—Our results suggest that the loss of CD21 and the upregulation of CD95 occur as separate events during the development of B-cell dysfunction. The loss of CD21 is a marker of B-cell exhaustion induced in the absence of appreciable viral replication, whereas the upregulation of CD95 is tightly linked to persistent viral replication and its associated immune activation. Thus, these dysfunctional profiles potentially represent two functionally distinct states within the B-cell compartment.

Keywords

B-cell dysregulation; elite controllers; exhaustion markers; HIV infection; viremia

Introduction

Numerous phenotypic and functional perturbations in the B-cell compartment are associated with HIV infection [1,2], including a decrease in the frequency of CD27⁺ memory B cells [3]; an expansion of immature/transitional B cells [4]; the upregulation of CD38, CD70, CD86 and CD95; and the downregulation of CD22, CD25, BAFF-R and LAIR-1 [5,6]. The overexpression of CD95 (Fas) has been repeatedly identified as a marker of B-cell activation

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Conflicts of interest

The authors have no conflicting financial interests.

that can lead to Fas/FasL-mediated apoptosis [3,6,7], which is associated with a decrease in antigen-specific B-cell responses [8–10].

Recent data has demonstrated that a large fraction of B cells lose CD21 expression during HIV infection [11]. Interestingly, CD21^{low} B cells [12,13], particularly the CD19⁺CD10⁺CD21^{low} subpopulation [5], exhibit reduced proliferative potential and express more CD95. By contrast, suppression of viremia with HAART has been associated with increased CD21 expression on B cells [12] and decreased CD95 expression [14], suggesting that viremia itself may be driving these changes in the B-cell population. These data suggest that both the loss of CD21 and the upregulation of CD95 may represent important independent markers of B-cell dysfunction; however, few studies have examined whether the combined expression of these markers may provide additional insight into the mechanism underlying the B-cell dysfunction observed in HIV infection.

Interestingly, a rare subset of HIV-infected individuals, known as controllers, are able to durably control viral replication in the absence of HAART [15], providing a unique opportunity to examine HIV-infected associated dysregulation in the absence of active viral replication or its associated immune activation. Given that the majority of studies aimed at examining the mechanism underlying B-cell dysfunction have focused on chronic, progressive HIV infection, it is currently not known whether HIV-associated B-cell dysregulation occurs due to irreversible damage to the B-cell compartment during infection or whether it is driven by persistently high levels of viral replication.

Therefore, to define the potentially different pathways that underlie B-cell dysfunction, we examined B-cell phenotypic profiles in both progressive and nonprogressive HIV infection. Despite low or undetectable plasma viral loads in viremic and elite controllers, we observed a loss of CD21 expression on tissue-like B cells in these individuals, similar to the loss observed in untreated, progressive patients with a high viral load. Yet, these two groups of controllers had differential expansion of CD21^{low} activated memory B cells and differential expression of the activation marker CD95. These data suggest that CD21 and CD95 may mark two potentially distinct pathways of B-cell exhaustion/activation that are divergently modulated by persistent viral replication.

Methods

Patients and phenotypic characterization

Flow cytometry was performed on cryopreserved peripheral blood mononuclear cells (PBMCs) obtained from 15 HIV-1-negative donors (mean age, 31 years; 33% women), 15 elite controllers [mean viral load (VL), 63 copies/ml; mean CD4⁺ T-cell count, 914/μl; mean age, 51 years; 40% women], 15 viremic controllers (mean VL, 460 copies/ml; mean CD4⁺ T-cell count, 739/μl; mean age, 38 years; 20% women), 15 chronic-treated patients (mean VL, <50 copies/ml; mean CD4⁺ T-cell count, 732/μl; mean age, 48 years; 20% women) and 15 chronic, untreated, viremic patients (mean VL, 28,519 copies/ml; mean CD4⁺ T-cell count, 518/μl; mean age, 38 years; 27% woman). The elite controllers were defined based on a consistent plasma HIV RNA level below the limit of detection used (e.g., <75 copies/ml when tested using bDNA or <50 copies/ml when tested using ultrasensitive PCR) [16]. All patients provided informed consent. PBMCs were prestained with blue viability dye (Life Technologies, Carlsbad, California, USA) and then stained with anti-CD19-PE, anti-CD10-PE-Cy5, anti-CD27-APC-Cy7, anti-CD95-APC (BioLegend, San Diego, California, USA) and anti-CD21-Pacific Blue (Exbio Praha, Vestec, Czech Republic). At least 1×10⁶ events were acquired using a BD LSRII flow cytometer, and the data were analyzed using FlowJo software v8.8.6 (TreeStar Inc., Ashland, Oregon, USA).

Statistical analyses

All statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, California, USA). The differences in the expression of the phenotypic markers among the different groups and subsets were analyzed using Kruskal–Wallis tests. *P* values less than 0.05 were considered statistically significant.

Results

CD21^{low} B cells accumulate in HIV-infected patients irrespective of viral control

Recently, the loss of CD21 has been suggested to be a marker of a unique population of exhausted B cells that accumulate in chronic, viremic HIV-positive patients [2,11,13]. However, whether this exhausted B-cell population accumulates due to persistent antigenic stimulation and/or its associated immune activation or to some other irreversible change in the B-cell compartment is unclear. Therefore, we compared the frequency of CD21^{low} B cells in a group of chronically infected viremic patients with the frequency observed in two groups of individuals with undetectable viral loads: chronically infected patients on HAART and a group of controllers who spontaneously maintain undetectable viral loads. As expected [12,17], CD21^{low} B cells were expanded in chronically infected, untreated (CU) patients when compared with healthy controls (*P*<0.001, Fig. 1b). Similar to previous reports [17], we found that virologic suppression via HAART successfully reduced the frequency of CD21^{low} B cells in treated, chronically infected individuals, suggesting that the inhibition of viral replication and its associated immune activation may reverse some aspect of B-cell dysfunction. Interestingly, CD21^{low} B cells were significantly expanded in both viremic controllers and elite controllers despite low-level viral replication [1,18,19] (elite controller vs. negative, *P*<0.01; elite controller vs. chronic-treated patients, *P*<0.05; viremic controller vs. negative, *P*<0.001; viremic controller vs. chronic-treated patients, *P*<0.01; Fig. 1b), suggesting that alterations in B-cell phenotypes are not driven solely by persistently high levels of viral replication and that these changes are reversible with HAART.

CD21^{low} B cells in controllers display a tissue-like B-cell phenotype

CD19⁺CD10[−]CD21^{low} B cells can be divided into two subpopulations with distinct functional profiles: activated memory (CD27⁺) B cells and exhausted tissue-like memory (CD27[−]) B cells (TLM). As the latter subset expands during chronic HIV infection [5], we determined which particular subset expanded in the controllers. We hypothesized that while tissue-like memory cells accumulate in CU patients, the activated memory cells would be expanded in controllers, thereby providing durable control of HIV infection. Surprisingly, the distribution of B-cell subsets in elite controllers and viremic controllers was similar to the distribution observed in CU patients (Fig. 1c), with a dramatic expansion of CD21^{low} tissue-like B cells (CU vs. negative, *P*<0.001; elite controller vs. negative, *P*<0.001; viremic controller vs. negative, *P*<0.001;) and a contraction of naive B cells when compared with aviremic chronic-treated patients (CU vs. chronic-treated patients, *P*<0.001; viremic controller vs. chronic-treated patients, *P*<0.01; elite controller vs. chronic-treated patients, *P*<0.05). By contrast, activated memory B cells (CD27⁺CD21^{low}) were expanded in patients with active viral replication (CU patients and viremic controllers) when compared with the chronic-treated patients (CU vs. chronic-treated patients, *P*<0.001; viremic controller vs. chronic-treated patients, *P*<0.01). These data suggest that while active viral replication drives the expansion of activated memory B cells, untreated HIV infection is associated with the expansion of exhausted tissue-like B cells, irrespective of spontaneous control.

CD21^{low} B cells in elite controllers are less activated than B cells in other HIV-positive patients

Previous reports have demonstrated that CD95/Fas is a marker of B-cell activation and that CD95 can directly contribute to B-cell dysfunction via the induction of apoptosis in activated B cells [5,7,14]. However, as little is known regarding the relationship between CD95 and CD21 expression, we addressed whether the combined expression of these two markers could provide additional insights into qualitative differences in the level of B-cell exhaustion occurring during HIV infection. The overall frequency of CD19⁺CD10⁻CD95^{high} B cells in CU patients was increased compared with healthy controls ($P<0.001$; Fig. 2a), whereas interestingly, viremic controllers exhibited an intermediate increase in the frequency of CD95^{high} mature B cells, and no increase was seen in elite controllers and chronic-treated patients. Combined, these data suggest that low-level viremia can drive CD95 expression on total B cells. To understand the differential regulation of Fas on different B-cell subpopulations, we examined CD95 expression in all patient cohorts. As expected, naive B cells displayed the lowest degree of activation (Fig. 2b), whereas increasingly higher Fas expression was seen on TLM and resting memory B cells, with the highest levels of expression detected on activated memory B cells. Interestingly, CD95 expression was always increased on activated memory B cells, irrespective of patient population, even in healthy controls (Fig. 2), suggesting that CD95 may mark a population of dysfunctional or aging B cells that are invariably destined for deletion. However, in CU patients, the frequency of CD95^{high} B cells in all subsets was significantly higher when compared with healthy controls (Naive and resting memory, $P<0.001$; TLM, $P<0.01$; activated memory, $P<0.05$), suggesting that virus-induced immune activation results in a generalized CD95 upregulation on all B-cell subsets. Furthermore, the activation of activated memory and resting memory decreased following HAART; although it did not return to the levels seen in HIV-negative individuals.

For all groups of HIV-infected patients, activated memory B cells had the highest proportion of activated, CD95^{high} cells. Interestingly, although the activated memory B-cell subset in elite controllers was not significantly expanded when compared with CU patients (Fig. 1c), the proportion of activated, CD95^{high} activated memory cells in elite controllers was significantly reduced when compared with CU patients (elite controller vs. CU, $P<0.001$; Fig. 2), suggesting that activated memory B cells may be protected from deletion in elite controllers. Similarly, although the proportion of TLM B cells was similar in elite controllers and CU patients (Fig. 1c), the fraction of activated, CD95^{high} TLM B cells in elite controllers was significantly reduced when compared with the CU patients (elite controller vs. CU, $P<0.01$; Fig. 2). Intriguingly, based on both their increased frequency of activated memory B cells and their increased expression of CD95, viremic controllers appeared to have a higher degree of B-cell activation, suggesting that even low-level viremia can drive B-cell activation. Taken together, these data suggest that although B-cell exhaustion in HIV infection is not linked to the level of viremia, B-cell activation, CD95 expression, and their related dysfunction is linked to the level of viral replication. Moreover, the data suggest that elite controllers have an expanded population of activated B cells that, due to the low expression of CD95, may be protected from deletion.

Discussion

Little is known regarding the mechanism(s) underlying HIV-associated B-cell dysfunction. Accumulating studies have suggested that several markers, including CD95 and CD21, are differentially expressed on B cells during progressive HIV infection and may mark exhausted and/or dysfunctional B cells [2,7,11–14]. However, because most of these studies focused on progressive infection, it is unclear whether high levels of active viral replication or infection-induced permanent damage within the B-cell compartment is responsible for

these changes. To address this question, we compared CD95 and CD21 expression in chronically infected patients and spontaneous controllers.

The expansion of CD21^{low} B cells during HIV-1 infection has been previously associated with high plasma viral loads, chronic immune activation and disease progression [20]. However, we observed an equivalent loss of CD21 expression in both chronically infected patients and controllers, suggesting that CD21 expression may be altered independently of immune activation or viral load. Moreover, similar to CU individuals, HIV controllers had a high frequency of exhausted, tissue-like B cells despite having low to undetectable viral loads. This suggests that the loss of CD21 expression is not caused exclusively by a high viral load and that the accumulation of dysfunctional CD21^{low} B cells may be linked to intrinsic infection-induced alterations in the B-cell compartment. Previous studies have demonstrated that alterations in B-cell subsets can occur with age [21]. However, even though there are significant differences in the ages of the various HIV-infected populations (elite controller vs. CU and elite controller vs. viremic controller, $P<0.05$), there was no correlation between patient age and either CD95 expression on bulk B cells or B-cell subsets or the expression of CD21 (data not shown). These data suggest that immunosenescence alone cannot account for the accumulation of exhausted tissue-like B cells in elite controllers.

CD95^{high} activated memory B cells were significantly expanded in all patient groups (Fig. 2), strongly suggesting that CD95 marks a population of activated B cells in both HIV-infected and healthy individuals. However, the frequency of CD95^{high} activated memory B cells was significantly lower in elite controllers when compared with CU individuals (Fig. 2), potentially reflecting an ability to protect B cells from deletion in elite controllers. These results demonstrate that, although CD21^{low} tissue-like B cells may represent an exhausted B-cell population that accumulates regardless of detectable viral replication, CD21^{low} activated memory B cells may represent an activated B-cell population that has not been driven to full activation and dysfunction in the absence of active detectable viral replication.

Elite controllers and viremic controllers are two distinct cohorts of rare individuals [15,16,18,19] who are able to spontaneously and durably control HIV replication in the absence of HAART. As elite controllers have undetectable VLs and viremic controllers have slightly higher VLs (<2000 copies/ml), our data suggest that CD95 upregulation and the expansion of activated memory cells occurs even in the presence of low-level, but detectable, viral replication. Thus, CD95 and CD21 highlight two divergent patterns of B-cell exhaustion, and the differential expression of these markers is associated with differential control of infection. Together, these data indicate that B-cell dysregulation in HIV infection is induced by a series of cumulative changes that damage the B-cell compartment.

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G.S. and G.A. conceived the study. G.S., A.E.M. and N.T. designed and performed the experiments. G.S., A.E.M. and T.S. analyzed the data. G.S., T.S. and G.A. wrote the article.

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Fig. 1. CD21^{low} B cells expand in all infected patient populations in the absence of therapy

(a) Gating strategy used to determine the frequency of CD21^{high} and CD21^{low} B cells in the mature CD19⁺CD10⁻ B-cell population. On the basis of the expression of CD27, the cells were divided into two B-cell subsets. Two representative dot plots from two patients are shown. (b) Frequency of CD10^{neg}CD21^{low} B cells among the different groups. (c) Relative proportion of B-cell subsets in the different populations: naive, CD27⁻CD21^{high}, tissue-like memory, CD27⁻CD21^{low}; resting memory, CD27⁺CD21^{high}; activated memory, CD27⁺CD21^{low}. The significance of the differences among the groups was assessed using Kruskal–Wallis tests. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Fig. 2. CD95 expression on B cells increases only in the context of detectable viral replication

(a) Frequency of total mature CD95^{high} B cells in all patient groups. (b) Frequency of CD95^{high} B cells in the different B-cell subsets in all patient groups. The data are presented as box-and-whisker plots, with the minimum to maximum value of each data set plotted. The significance of the differences among groups was assessed using Kruskal–Wallis tests. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.