Expansions of CD4+CD28– and CD8+CD28– T cells in Granulomatosis with Polyangiitis and Microscopic Polyangiitis Are Associated with Cytomegalovirus Infection But Not with Disease Activity

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ABSTRACT. ***Objective.*** T helper cells lacking CD28 (CD4+CD28–) have been implicated in the pathogenesis of granulomatosis with polyangiitis (Wegener; GPA) and microscopic polyangiitis (MPA). Expansions of CD4+CD28– and CD8+CD28– T cells have also been associated with latent cytomegalovirus (CMV) infection. We assessed these T cells with and without coexpression of CD56 and CD57 in relation to vasculitis as well as CMV status.

Methods. Blood from 16 patients in remission (12 GPA, 4 MPA), 18 patients with active vasculitis (12 GPA, 6 MPA), and 20 healthy controls was examined by flow cytometry for expression of CD4, CD8, CD56, CD57, and CD28 on T cells. The influence of age, CMV status, presence of disease, and disease activity on T cell subpopulations was tested with multiple regression analyses.

Results. In active vasculitis, the total numbers and proportion of lymphocytes were decreased. Total numbers of CD4+, CD8+, CD4+CD28–, CD8+CD28–, CD4+CD57+, and CD8+CD57+ T subpopulations were decreased to the same extent, implying unchanged proportions. Multivariate analyses showed no associations between vasculitis and CD28– or CD57+ T subpopulations, whereas immunoglobulin G antibodies to CMV were associated with expanded proportions of CD28– and CD57+ T cells, in both the CD4+ and the CD8+ compartments.

Conclusion. CD28– and CD57+ T cells were associated with latent CMV infection and not with a diagnosis of GPA or MPA. Vasculitis assessment should include CMV status. (J Rheumatol First Release Aug 1 2012; doi:10.3899/jrheum.120060)

Key Indexing Terms:
VASCULITIS T CELL CD28 CD56
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Accumulations of CD8+CD28– and CD4+28– T cells have been reported in patients with the antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (Wegener; GPA)[1]. These T cell subpopulations are also expanded in inflammatory diseases such as rheumatoid arthritis (RA), but also in normal aging, as well as in cytomegalovirus (CMV) and human immunodeficiency virus infections[2,3,4,5].

T cells lacking CD28 often coexpress CD57[2,3]. CD56 is a natural killer (NK) cell marker also expressed on subpopulations of T cells — NKT-like cells[6,7,8]. Expression of both CD56 and CD57 on CD8+ cells has been associated with CMV exposure[9].

Our aim was to assess CD4+ and CD8+ T cells for their expression of CD28, CD56, and CD57, and relate the findings to GPA/MPA, age, and CMV infection.

MATERIALS AND METHODS

Participants comprised 16 patients with GPA or MPA[10] in remission (median age 75 yrs, 7/16 men, GPA/MPA: 12/4), 18 patients with active vasculitis (median age 67 yrs, 12/18 men, GPA/MPA: 12/6), and 20 healthy controls (median age 70 yrs, 12/20 men). PR3– and myeloperoxidase-ANCA were positive in 24 and 9 patients, respectively (1 unknown). Clinical characteristics of individual patients including Birmingham Vasculitis Activity Score (BVAS) were reported previously[11]. GPA was restricted to upper airways in

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2/12 in the remission group and 3/12 in the active group. Methylprednisolone pulses were given in 4 and prednisolone 2.5–80 mg/day in 12 patients with active vasculitis, while prednisolone at doses of 0–5 mg/day were used in the remission group. The study was approved by the regional ethics committee.

Blood samples were analyzed directly by 6-color flow cytometry using monoclonal antibodies to CD3 (clone SK7), CD4 (clone SK3), CD8 (clone SK1), CD56 (clone NCAM 16.2), CD45 (clone 2D1), CD57 (clone HNK-1), and CD28 (clone L293; BD Biosciences, San Jose, CA, USA), as described[11]. Immunoglobulin G (IgG) antibodies to CMV were analyzed with a chemiluminescent microparticle immunoassay (Abbott Laboratories, Chicago, IL, USA).

The Kruskal-Wallis (KW) test was used, and the Mann-Whitney (MW) U test if $p < 0.05$, to compare multiple and 2 groups, respectively. Median and interquartile ranges (IQR) are given. Spearman correlation analysis was used for continuous variables. The influence of CMV infection, age, and vasculitis on T cell subpopulations was assessed with multivariate regression analysis.

RESULTS

In active vasculitis, the number of leukocytes increased [median 12.9 (IQR 9.8–14.5) × 10^9 cells/l] compared with remission [median 6.8 (IQR 5.5–8.4) × 10^9 cells/l] and controls [5.4 (IQR 3.7–7.9) × 10^9 cells/l; KW $p < 0.0001$], whereas the number of lymphocytes decreased, causing a reduced proportion of lymphocytes [active: 10% (3.9%–16%), remission: 26% (16%–35%), controls: 36% (32%–43%); KW $p = 0.0007$].

The proportions of CD4+ and CD8+ T cell subpopulations with regard to CD 57 and CD28 did not differ between active vasculitis, remission, and controls, whereas the proportion of CD3+CD56+ NKT-like cells was lower in active vasculitis compared with controls [active: 3.4% (1.9%–9.2%), remission: 12% (4.4%–16%), controls: 8.0% (5.4%–14%); MW $p = 0.018$, KW $p = 0.046$]. The majority of CD3+CD56+ cells expressed CD8+, which was lower in active vasculitis compared with remission [active: 8.5% (2.3%–17%) of CD8+ cells, remission: 19% (9.6%–30%; MW $p = 0.041$), controls: 14% (11%–25%)].

CD4+CD28– T cells were studied regarding CD56 and CD57 expression. There was a decreased proportion in the active group of CD4+CD28–CD56+ T cells [active: 2.7% (0.0%–20%), controls: 28% (6.0%–41%; MW $p = 0.017$, KW $p = 0.044$), remission: 7.2% (1.1%–44%); not significant]. Further, the subgroup of CD4+CD28– T cells expressing both CD56+ and CD57+ was also lower in active vasculitis compared with controls [active: 1.4% (0.0%–18%), controls: 22% (4.0%–38%; MW $p = 0.011$, KW $p = 0.036$), remission: 4.8% (1.1%–37%); not significant]. For CD8+CD28– T cells, CD56 and CD57 expression did not differ between the clinical groups. CD28– T cells (both CD4 and CD8) were highly correlated to both CD57+ T cells ($r = 0.933$, $p < 0.00001$) and CD56+ T cells ($r = 0.657$, $p < 0.00001$).

Anti-CMV IgG antibodies were found in 68% of patients with vasculitis (remission: 69%, active: 67%) and in 90% of controls (nonsignificant difference). After univariate analyses (Table 1), age-adjusted multiple regression analysis confirmed that CMV was independently related to CD8+CD28–, CD4+CD28–, CD8+CD57+, and CD4+CD57+ T cells (Table 2). A similar independent relationship between age and T cell subpopulations was found. In contrast, vasculitis was not related to any of these T cell subpopulations. In univariate analysis, disease activity (BVAS) was not related to any of the T cell subpopulations (data not shown). Figure 1 illustrates that CMV, but not vasculitis, influences both CD8+CD28– and CD4+CD28– T cells.

DISCUSSION

In our study, latent CMV infection was strongly associated with expansions of CD28– and CD57+ T cells, both in the CD4 and in the CD8 compartments. Conversely, a diagnosis of vasculitis was unrelated to CD28– and CD57+ T cells. A recent article reported that expansion of circulating CD4+CD28– T cells of patients with GPA was driven by CMV infection[12]. Our data agree, and extend the association also to CD8+CD28– T cells.

The CD4+CD28– population is small compared to the CD8+CD28– population. Cytotoxic CD8+ T cells are crucial in viral defense and go through several steps of differentia-

Table 1. Proportions (median % and interquartiles) of CD56+, CD57+, and CD28–CD8+ T cells (left panel) and CD4+ T cells (right panel) in relation to cytomegalovirus (CMV) status in the whole population of patients and controls. In these univariate analyses, latent CMV infection was associated with CD8+CD28– and CD4+CD28– T cells, and also with CD8+CD57+ and CD4+CD57+ T cells. In contrast, the proportions of T cells expressing CD56 did not differ across groups.

Proportion (%) of CD3 or CD8	CMV Pos	CMV Neg	p*	Proportion (%) of CD3 or CD4	CMV Pos	CMV Neg	p*
CD8+ % of CD3	26 (21–32)	21 (14–27)	0.159	CD4+ % of CD3	44 (33–51)	37 (29–45)	0.213
CD8+CD56+ % of CD8	14 (7.3–27)	9.6 (4.3–19)	0.223	CD4+CD56+ % of CD4	1.4 (0.3–3.2)	0.6 (0.2–0.8)	0.164
CD8+CD57+ % of CD8	34 (23–45)	18 (8.5–27)	**0.003**	CD4+CD57+ % of CD4	11 (8–20)	0.9 (0.6–1.9)	**0.0001**
CD8+CD28– % of CD8	44 (26–64)	19 (7.8–30)	**0.003**	CD4+CD28– % of CD4	5 (2–11)	0.1 (0.0–0.5)	**0.0009**

p values < 0.05 are in bold type. * Mann-Whitney U test.

Table 2. Multiple regression analysis was used to test any influence of vasculitis/controls, age, and cytomegalovirus (CMV) on the proportion (%) of different T cell subpopulations in the whole group of patients and controls. $p < 0.05$ was considered significant (indicated in bold type). As in univariate analyses, latent CMV infection (but not vasculitis) was associated with CD8+CD28– and CD4+CD28– T cells, and also with CD8+CD57+ and CD4+CD57+ T cells. In contrast, the proportions of T cells expressing CD56 did not differ across groups. Using the variable "active vasculitis versus remission" instead of "vasculitis versus controls" did not change the results (data not shown).

Proportion (%) of CD8+	Age, p	CMV+ vs –, p	Vasculitis vs Controls, p	Proportion (%) of CD4+	Age, p	CMV+ vs –, p	Vasculitis vs Controls, p
CD8+CD56+ % of CD8	0.528	0.303	0.763	CD4+CD56+ % of CD4	0.556	0.257	0.736
CD8+CD57+ % of CD8	**0.005**	**0.004**	0.746	CD4+CD57+ % of CD4	**0.010**	**0.002**	0.304
CD8+CD28– % of CD8	**0.023**	**0.003**	0.648	CD4+CD28– % of CD4	**0.047**	**0.005**	0.669

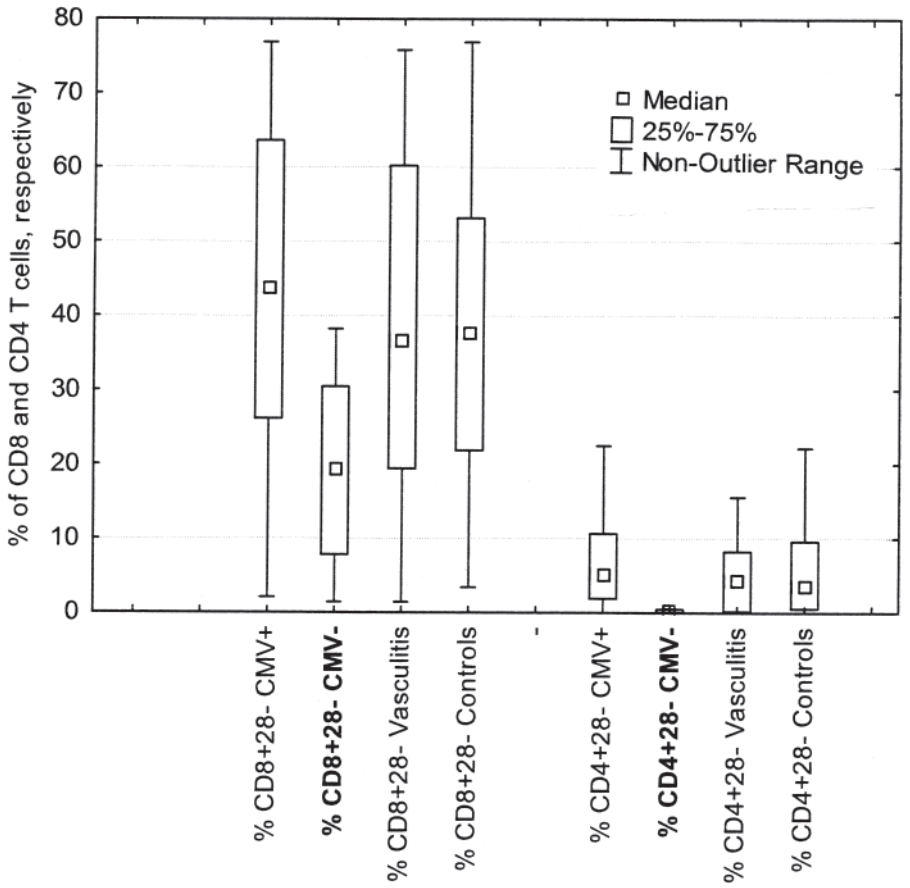

Figure 1. Significantly lower proportions of both CD8+CD28– and CD4+CD28– T cells are observed in a composite group of patients and controls without latent cytomegalovirus (CMV) infection, as reflected by negative antibodies of immunoglobulin G-type against CMV. CMV-negative patients and controls are indicated in bold type. Patients with vasculitis and healthy controls did not differ concerning CD8+CD28– or CD4+CD28– T cells.

tion: loss of CD28 and addition of CD57, followed by loss of CCR7 and switch from CD45RO to CD45RA[5,9]. As CD8+CD28– T cells increase with age, matching of patients and controls concerning both age and CMV status is important. In our material, age did not differ across groups, whereas CMV tended to be more common in controls (90%) than in patients (68%).

Unlike CD57, expression of CD56 was not associated with CMV status or age. Instead, CD56+ T cells were lower in the active group. CD56 is a marker of NKT-like cells, which con-

stitute a heterogeneous and sometimes immunoregulatory population[6]. One subgroup is the Vα24Vß11 NKT cells[7], which were decreased in patients with GPA in 1 report[13]. The precise role of CD56 expression on T cells needs further investigation.

We found that expanded CD28– and CD57+ T cells, in both the CD4 and CD8 compartments, were associated with latent CMV infection rather than a diagnosis of vasculitis. In contrast, T cells expressing CD56 were inversely related to active vasculitis.

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