

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
CYTOMEGALOVIRUS

T CELL
ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES

CD28
CD56

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 exposure⁹.

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 MPA¹⁰ 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 previously¹¹. 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 described¹¹. 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) $\times 10^9$ cells/l] compared with remission [median 6.8 (IQR 5.5–8.4) $\times 10^9$ cells/l] and controls [5.4 (IQR 3.7–7.9) $\times 10^9$ 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 infection¹². 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+	26	21	0.159	CD4+	44	37	0.213
% of CD3	(21–32)	(14–27)		% of CD3	(33–51)	(29–45)	
CD8+CD56+	14	9.6	0.223	CD4+CD56+	1.4	0.6	0.164
% of CD8	(7.3–27)	(4.3–19)		% of CD4	(0.3–3.2)	(0.2–0.8)	
CD8+CD57+	34	18	0.003	CD4+CD57+	11	0.9	0.0001
% of CD8	(23–45)	(8.5–27)		% of CD4	(8–20)	(0.6–1.9)	
CD8+CD28–	44	19	0.003	CD4+CD28–	5	0.1	0.0009
% of CD8	(26–64)	(7.8–30)		% of CD4	(2–11)	(0.0–0.5)	

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

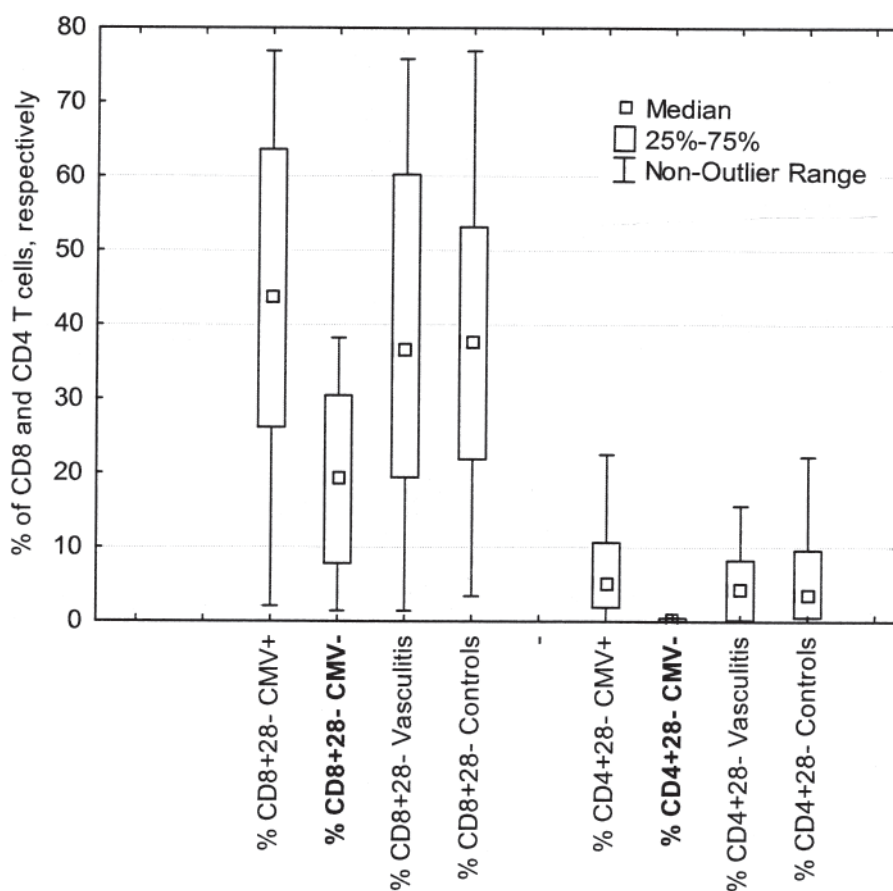


Figure 1. Significantly lower proportions of both CD8+CD28- and CD4+CD28- T cells are observed in a composite group of patients and controls without latent cytomegalovirus (CMV) infection, as reflected by negative antibodies of immunoglobulin G-type against CMV. CMV-negative patients and controls are 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, where-

as 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 population⁶. One subgroup is the V α 24V β 11 NKT cells⁷, which were decreased in patients with GPA in 1 report¹³. 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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