Expression of CD57 on CD8⁺ T lymphocytes of patients with Wegener's granulomatosis and microscopic polyangiitis: evidence for continuous activation of CD8⁺ cells

C. Iking-Konert¹, T. Vogl¹, B. Prior², E. Bleck¹, B. Ostendorf¹, K. Andrassy³, M. Schneider¹, G.M. Hänsch²

¹Rheumatology, Department of Endocrinology, Diabetology and Rheumatology, Heinrich-Heine University of Düsseldorf, Germany; ²Institute for Immunology, Ruprecht-Karls University of Heidelberg, Germany; ³Medizinische Klinik, Ruprecht-Karls University of Heidelberg, Germany.

Christof Iking-Konert, PD Dr. Med Tim Vogl Birgit Prior Ellen Bleck Benedikt Ostendorf, PD Dr. Med Konrad Andrassy, Prof. Dr. Med Matthias Schneider, Prof. Dr. Med

G. Maria Hänsch, Prof. Dr. Please address correspondence to: Dr. med. Christof Iking-Konert, Rheumatology, Department of Endocrinology, Diabetology and Rheumatology, Heinrich-Heine-University Moorenstr 5, 40225 Düsseldorf, Germany.

E-mail: christof.iking-konert@roche.com Received on March 18, 2008; accepted in revised form on August 29, 2008.

Clin Exp Rheumatol 2009: 27 (*Suppl. 52*): *S19-S24*.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2009.

Key words: ANCA, vasculitis, T cells, T lymphocyte, CD57, CD8, Wegener's granulomatosis, AAV.

Abbreviations:

ANCA:	anti-neutrophil cytoplasmic
BVAS:	Birmingham vasculitis activity
CD:	score cluster of differentiation
CMV:	cytomegalovirus
EBV:	Epstein-Bar-virus
FITC:	fluorescein isothiocyanate
IFN:	interferon
IL:	interleukin
MHC:	major histocompatibility complex
MPA:	microscopic polyangiitis
PE:	phycoerythrine
PMN:	polymorphonuclear neutrophils
PR3:	proteinase 3
WG:	Wegener's granulomatosis

Competing interests: none declared.

ABSTRACT

Objectives. To gain insight into the immune pathogenesis of Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA), the prevalence of circulating CD8⁺ T lymphocytes expressing CD57 as a marker for previous activation was analyzed.

Methods. Receptor expression of CD57 was measured in CD8⁺ T cells of patients with active disease (n=5) by cytofluorometry and compared with expression in patients in remission (n=80) and in age-matched healthy donors (n=34). The results were compared to clinical parameters including severity and duration of the disease.

Results. CD8⁺CD57⁺ were detected in patients with WG and MPA and in healthy donors as well and increased considerably with age. Compared to age-matched healthy donors, the prevalence of CD8+CD57+ was increased in the younger patients (up to 40 y). In most patients a high percentage of CD8+CD57+ coincided with severe disease and multiple organ involvement, while low CD8+CD57+ percentage was seen in patients with limited disease or in patients in complete remission. In patients with smoldering disease, the percentage of CD8⁺CD57⁺ increased with time. High numbers of CD8+CD57+ correlated with low CD4:CD8 ratio.

Conclusions. In patients with WG and MPA a population of $CD8^+/CD57^+$ expand, identifying terminally differentiated $CD8^+$ cells. The prevalence of $CD57^+$ cells was related to the course of disease. So far, the function of CD57 on $CD8^+$ cells is not understood. However, these cells might produce certain cytokines, which play a role in the pathogenesis of AAV. The data support the hypothesis that $CD8^+$ T cells are activated in the context of primary vasculitides.

Introduction

Activation of T lymphocytes is generally studied in the context of virus infection. There is, however, unequivocal evidence for T cell activation in chronic inflammatory diseases, including rheumatoid arthritis, lupus erythematosus, or ANCA-associated primary vasculitis (AAV), such as Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA). Because these patients often require long-term immunosuppressive therapy, clinically inapparent virus infections as a cause of T-cell activation are difficult to rule out. On the other hand, data derived from patients with newly diagnosed disease indicated activation and expansion T-cell clones.

T-cell activation can be monitored by loss of surface receptors, and by acquisition of activation-associated surface antigens, *e.g.* acquisition of CD11b by CD8⁺ T cells was found in patients with active WG (1-3). Following activation, CD8⁺ T cells persist, and hence accumulate, while CD8⁺ cells undergo apoptosis following activation. Accordingly, in patients with WG, a loss of the absolute number of CD8⁺ cells is seen, accompanied by a shift of the CD4 to CD8 ratio towards CD8; and a prevalence of CD8⁺ cell lacking the costimulatory receptor CD28 (3-5).

In the present study, we assessed the expression of CD57 on T cells of patients with WG and MPA as compared to healthy donors. CD57, originally called HNK-1, is a glycoprotein found on subsets of NK cells and also of T lymphocytes. For the latter, is thought to identify terminally differentiated T cells, particularly CD8⁺ cells in the state of replicative sencescence [6]. In line with this notion, increasing numbers of CD57⁺ T cells are found in patients with persistent viral infections including Epstein Virus, cytomegalo-

Expression of CD57 on CD8⁺ T cells in patients with primary vasculitis / C. Iking-Konert et al.

virus or HIV-1 infections, with chronic inflammatory disease, and in the elderly (6-9). As terminally differentiated cells, CD57+ T cells do not proliferate, they, however, express cytotoxic entities, and are able to produce cytokines, particularly gamma interferon (8, 10). We here describe that CD57 is expressed by CD8+ cells of patients with active WG, and of healthy donors as well. The prevalence of CD8+CD57+ depended predominantly on the age. Only in patients with severe disease and multiple organ involvement a trend towards a proportion of CD8+ CD57+ was seen.

Patients, material and methods

Patient characteristics

The study was approved by the ethics committees of the Universities of Heidelberg and of Düsseldorf, respectively. After having obtained informed consent, between 1999-2006, 90 patients were recruited by the renal unit of the University of Heidelberg University Hospital and the Department of Rheumatology/ University of Düsseldorf. Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) were classified according to the definition of the Chapel Hill conference (11) and to the ACR criteria for the classification of WG (12). Five patients had active disease with a BVAS >5 (according to the Birmingham vasculitis activity score, (13)). Of these patients four had untreated, newly diagnosed (WG), 1 patient had a relapse after remission for 2 years. Eighty patients (47 with WG and 33 with MPA) in remission (BVAS <1) for one year or more were also included. The patients in remission were divided into the following groups: those with long standing disease (>5 years, duration of disease was determined as time elapsed since the first diagnosis) and those with disease duration of 5 years or less. Moreover, the patients were grouped according to severity of the disease. "Non-severe" (S0) was defined as no disease activity within the last 5 years (n=7); "less severe" (S1) was defined as follows: disease without major organ involvement and <1 relapse (n=12); "severe disease" (S2) was defined as follows: systemic disease including at least one major organ (e.g. kidney, lung, central nervous system (CNS), and history of more than 1 relapse (n=18). Localized disease was limited to manifestations of the head (*e.g.* ear, eye and nose, excluding CNS, n=6). Furthermore, the cumulative cyclophosphamide dose was calculated for each single patient. Patients were grouped by the number of organs involved, as described by de Groot *et al.* (14). For comparison, blood of healthy donors (n=34), matched with regard to age, was drawn, after having obtained informed consent and observing the institutional guidelines.

Cytofluorometry

The expression of CD8, CD11b, CD28, and CD57 was measured in whole blood by FACScan using standard procedures. Cells were triple-stained using phycoerythrine (PE) or APC-labelled antibodies to CD8 (Becton Dickinson, BD), CD28 (Serotec) or CD11b (Biozol), fluorescein isothiocyanate (FITC)labelled antibodies to CD28 (Serotec Düsseldorf, Germany), or CD57 (BD). The isotype controls IgG1, IgG2a, IgG2b were used in the same final concentration, and label, respectively. To determine the CD4 to CD8 ratio, a mixture of anti-CD4 FITC- anti-CD8-PE was used with IgG FITC/ IgG PE as control (all antibodies obtained from Beckman Coulter, Marseille).



Fig. 1. Expression of CD57 on CD8⁺ T cells of patients and healthy donors: A upper panel: peripheral blood cells were labelled with antibodies to CD8 (APC), CD28 (PE), and CD57 (FITC). The gate was set for the CD8⁺ cells, and expression of CD57 and CD28 was measured. Shown are cells of two healthy donors (D1, D2), and two patients (P45, P66) in remission. In the donors, the CD57 expression increased with age.

B: Ten donors in the age range of 25 to 40 years (median 36 y) are shown, and 27 donors of 55 to 75 years (median 68) (open boxes). The patients (striped boxes) were age-matched patients (median age 36, n=9; median age 68, n=46). A significant difference between patients and donors regarding the CD8⁺CD57⁺ cells was only found for the age group 36 y (calculated by Mann-Whitney Wilcoxon test).

C CD57 was mainly associated with CD8⁺CD28⁻ cells; there was, however, a close correlation between CD8⁺CD28⁺CD57⁺ and CD8⁺CD57⁺ cells (D).

Expression of CD57 on CD8⁺ T cells in patients with primary vasculitis / C. Iking-Konert et al.

Data analysis

For technical reasons not all parameters were measured in all patients or donors. The number of individuals that were compared with regard to one particular parameter is given in the figure or the figure legend, respectively. The results of the FACScan data are expressed as "% positive" cells in the respective gate in relation to CD8+ cells. Differences between groups were calculated by Wilcoxon Mann Whitney U-test; differences between mean values by ANOVA one-way. Chi square test was used to analyse the coincidence of low CD4 to CD8 ratio and the expression of CD57 on CD8⁺ or CD8⁺CD28⁺ cells.

Results

Using cytofluorometry, CD8⁺CD57⁺ were detected in patients with WG and MPA as well as in healthy donors. The expression of CD57 varied widely

Fig. 2. Analysis of activation parameters on T cells of a patient with newly diagnosed disease without therapy: Peripheral blood cells were labelled with antibodies to CD8 (APC), CD28 (FITC or PE), CD11 (PE), and CD57 (FITC). The gate was set for the CD8 cells. At time of diagnosis and prior to immunosuppressive therapy, a small but conspicuous population of CD8+CD28+CD11b+ and CD8+CD28+CD57+ T cells was detected (upper panel). After immunosuppressive therapy (cyclophosphamid and corticosteroids), the percentage of CD28+CD57+ cells decrease, while CD28⁻CD57⁺ cells increased, and eventually also the population CD8+CD28+CD11b+ cells declined. Concomitantly, CD28-CD11b+ and CD28-CD57 cells appeared.

among the patients and the donors, and increased considerably with age. Compared to age-matched healthy donors, the prevalence of CD8⁺CD57⁺ was increased in the younger patients (up to 40 y). In the other age groups, there were no differences between the patients and the healthy donors (an example is shown in Fig. 1A, summary of all patients in Fig. 1B).

As seen in Fig. 1C, CD57 is expressed by CD8⁺CD28⁺ cells, and CD8⁺CD28⁻ cells. In all age groups, CD8⁺CD28⁻CD57⁺ were prevalent in patients and in donors as well (patients CD8⁺CD28⁺CD57⁺ 15.5 \pm 16.5 %; CD8⁺CD28⁻CD57⁺ 56.8 \pm 19.1%; donors CD8⁺CD28⁻CD57⁺ 17.7 \pm 9.0; CD8⁺CD28⁻CD57⁺ 44.5 \pm 14.0; values given as mean \pm SD of n=75 (patients) or n=34 (donors)). Again only for the group of patients of 40 years and younger, a higher percentage of

CD8⁺CD28⁺CD57⁺ cells was calculated (25.1 \pm 15.2% versus 15.2 \pm 8.5; mean \pm SD; *p*=0.029). There was a reasonable good correlation between the percentage of CD8+CD57+ and either CD8⁺CD28⁺CD57⁺ or CD8⁺CD28⁻ CD57⁺ (r=0, 78; Fig. 1D).

CD57 expression on CD8⁺ cells of

patients with newly diagnosed disease The data so far suggested an up-regulation of CD57 in the course of WG and MPA which, most likely, was obscured by the age-dependent increase in CD8⁺CD57⁺, therefore we did follow up-studies in patients with newly diagnosed WG (n=4). In all, active disease was associated with an expansion of CD8⁺CD28⁺CD11b⁺ cells (Fig. 2A). Expression of CD57, in contrast, was within the range of that of healthy age-matched controls (example in Fig. 2A), but unlike in healthy donors or





patients in remission, the prevalence of CD8+CD28+CD57+ was higher than that of CD8+CD28- CD57+ cells. Moreover, CD57 was also found on CD8+CD11b+ T cells, while in healthy donors CD8+CD11b+CD57+ is well below 5% of total CD8+. After onset of the immunosuppressive therapy with cyclophosphamide and corticosteroids, the proportion of CD28+CD57+ cells declined, and concomitantly CD28⁻CD57⁺ increased, as did the percentage of CD11b+CD57+ double positive cells (Fig. 2). Of note, the expansion of CD8+CD28+CD11b+ cells did not cease within the first 4 weeks after immunosuppressive therapy, compatible with the kinetics of the immunosuppressive effect of cyclophosphamide on T cells. The follow-up studies for 3 other patients, and for one patient with a relapse 2 years after remission gave essentially similar results. Of note, when all patients in remission for 1 year or more were considered we found that the percentage of CD8+CD11b+CD57+ was significantly higher in the patients when compared to healthy donors (14.1±9.5% for the patients and 7.48 + 4.2% for the donors; p=0.038).

$CD8^+CD57^+$ T cells in

patients in remission

To determine whether the prevalence of CD57+ cells was related to the course of disease, the patients were grouped according to the following parameters: duration of disease (determined as time elapsed since the first diagnosis), severity, systemic versus localised disease, number of organs involved, and the duration and extent of immunosuppressive therapy with cyclophosphamide.

We found that patients with long-standing disease (4 y and more) had a marginally significant higher percentage of CD8+CD57+ cells compared to patients with disease for less than 4 years (p=0.048, according to Wilcoxon Mann Whitney test). Again, only marginal differences were seen with regard to the prevalence of CD8+CD57+ when patients with severe disease were compared to patients with limited or oneshot disease (p=0.034; Fig. 3A and B). On the other hand, patients with a prevalence of CD8+CD57 positive cells greater than 20% (this is the





The patients were group according to A: severity of disease (S0: "Non-sever": no disease activity within the last 5 years; n=7); S1: "less severe" disease without organ involvement and <1 relapse (n=12); S2: "severe disease" with systemic manifestation including at least one major organ (*e.g.* kidney, lung, central nervous system (CNS)), and history of >1 relapse; n=18).

B: systemic or localised disease (limited to manifestations of the head (*e.g.* ear, eye and nose, excluding CNS, n=6)) or C number of organs involved (adapted to Groot *et al.*). Each dot (O) represents the percentage of CD8⁺CD57⁺ cells of one patient, the bars (-) show the median values. Differences between groups were calculated using the Mann-Whitney Wilcoxon test. The differences are calculated against Group 1.

average calculated for all patients), were found predominantly in the group of patients with severe disease (S2; 11 of 28 patients; 39.3%), to a lesser extent in the group with less severe disease (S1; 5 of 21 patients; 23.8%), and none in the group of patients with disease-free intervals for 5 years or more, and without therapy (S0; n=7). The same was true for CD8⁺CD57⁺ cells: 60% of the patients with severe disease had more than 30% of CD8⁺CD57⁺, but only 37% of the patients with less severe disease. Of the patients with disease-free intervals for five years and more, one patient (1 of 7) had 41% CD8⁺CD57⁺ cells. When patients with localised (*e.g.* manifestation of the head excepts CNS) versus systemic disease were evaluated, a high percentage of CD8⁺CD28⁺CD57⁺ and CD8⁺CD57⁺ cells was found in one of seven patients with localised disease versus 14 of 48 (CD8⁺CD28⁺CD57⁺) Fig. 4. CD8+CD28+CD57 CD8+CD28+CD11b+ and expression on T cells in the time course of disease: In patients with smoldering disease or relapse, the percentage of CD28+CD11b+ CD28+CD57+ and cells raised simultaneously n=5) (closed symbols. while the percentage of these cells remained stable in well controlled disease (open symbols, n=3). Correlation between B: CD57 on CD⁺ cells and the CD4/CD8 ratio. The distribution is significant (using the Chi square test, p=0,001, 10,02) when the

p=0,001, 10,02) when the cut off values for the CD4/ CD8 ratio of 1.5 and for CD8/CD57 of 30% were used (normal values for healthy controls).



or 18 of 48 (CD8⁺CD57⁺) for patients with systemic disease. CD57 expression increased with the number of organs involved (Fig. 3C). No correlation at all was seen between CD57 expression and the cumulative cyclophosphamide dose.

Follow-up studies of selected patients revealed that in patients with smoldering, active disease (n=5), as opposed to patients with well-controlled disease (n=3), the percentage of CD8+CD28+CD57+ increased (Fig. 4A), in parallel with an increase of CD11b positive cells, and a shift of the CD4 to CD8 ratio towards CD8. When all patients were considered CD11b expression did not correlate with CD57 expression; a high percentage of CD57 positive cells, however, coincided with low CD4 to CD8 ratio (Fig. 4B).

Discussion

The aetiology and pathogenesis of WG and MPA is still elusive. WG usually starts as a so-called localized disease with granulomatous lesions of the respiratory tract before it converts to a generalized, systemic disease (15). There is convincing evidence for an activation of T cells in the early course of disease (1-3, 16). Lack of CD28 expression on peripheral blood CD4⁺ as well as on CD8⁺ T cells has been reported in patients with WG earlier, suggesting a pathogenetic role of CD28⁻ T cells in WG. Increasing numbers CD4⁺/ CD28⁻ T cells have been described and identified as a major source of proinflammatory cytokines (such as IFN- γ and TNF- α) mediating granuloma formation and cytotoxicity and are enriched in the lung of patients with WG (17, 18).

In patients with long-lasting disease and immunosuppressive therapy for an extended period of time, in contrast, it is difficult to attribute alterations within the T-cell compartment to progression of the underlying disease or to the therapy, which is essentially directed to lymphocytes. In the recent years, however, the increasing knowledge of the activation and the fate of T cells following activation enable us to discriminate between mere cytotoxic effects, and activation-associated alterations. Thus, it became clear that CD8⁺ T cells persist following activation, and form a pool of terminally differentiated cells, while CD4+ cells are eliminated following activation (19-20). Accepting these notions, the most pertinent observations with regard to the T cells in primary, ANCA-associated vasculitis (WG and MPA), such as the shift of the CD4 to CD8 ratio towards CD8, the loss of CD4+ cells, and the prevalence of CD8+CD28- cells could be explained as consequence of activation, differentiation and the ensuing replicative senescence within the CD8⁺ compartment (4-5).

To assess terminal differentiation within the CD8⁺ compartment more directly, we determined the expression of CD57 on peripheral blood cells of patients with ANCA-associated vasculitis. As published previously by others, the prevalence of CD8+CD57+ increases with age (21), presumably as the result of continuous and/or repeated activation as it will occur during the immune response, particularly in response to virus infections such as cytomegalia (CMV). Antigen-specific CD8⁺ cell clones expand, differentiate to effector cells, and eventually reach the terminal stage, which is characterised by expression of CD57, an observation that was confirmed recently by others (22). When analysing our patients with newly

diagnosed disease, we found essentially a similar differentiation of CD8+: during active disease CD8+ cell expand, identified by expression of CD11b on the CD8+CD28+ cells. CD8+CD28+CD11b+ are a transient phenotype that gives rise to CD8+CD28-CD11b+, which eventually also acquired CD57. Since the latter persist, they accumulate following immunosuppressive therapy. Of note, CD8+CD28+CD11b+ cells do not decline immediately following immunosuppressive therapy, in contrast to activated PMN, as we had shown before (2-3). CD8⁺ cells co-expressing CD11b and CD57 were found also in patients in remission. We presume that these are rem-

Expression of CD57 on CD8⁺ T cells in patients with primary vasculitis / C. Iking-Konert et al.

nants of previous disease-associated activation of CD8⁺, because in healthy donors CD8⁺ cells co-expressing CD11b⁺ and CD57⁺ are rather infrequent.

The prevalence of CD8+CD57+ cells, however, was not different when patients and healthy donors were compared. This observation, however, is not inconsistent with a disease-associated activation of CD8+ because CD8+CD57+ are also generated in the context of an immune response to infection in both, patients and donors, and thus contribute to the pool of CD8+CD57+ cells. The persistence of these cells explains not only the increase of CD8+CD57+ cells with age, but also the wide variation which reflects the individual frequency of infections. The presence of CD8+CD57+ cells that have been generated in response to infection might obscure the increase of CD8+CD57+ occurring in the course of vasculitis. The observation, that in patients up to 40 years the prevalence of CD8+CD57+ cells is higher when compared to the age-matched donors supports this assumption: in younger individuals with less frequent exposure to infectious agents, the CD8+CD57+ pool is still smallish, so an increase due to an inflammatory or autoimmune event is more apparent.

That there is a linkage between CD8+CD57+ cells and WG/ MPA becomes also apparent when relating the percentage of CD8+CD57+ cells to clinical parameters. In the majority of patients a higher than average percentage of CD8+CD57+ coincided with severe disease and multiple organ involvement, while low CD8+CD57+ percentage was seen in patients with limited disease or in patients in complete remission for an extended period of time. Moreover, in patients with smoldering disease, the percentage of CD8+CD57+ increase with time, more or less in parallel with the expansion of CD8+CD28+CD11b+ cells, the presumed precursors of CD8+CD57+. That high numbers of CD8+CD57+ correlated with low CD4: CD8 ratio supports that notion, because a loss of CD4+ cells and a shift towards CD8⁺ is mainly seen in patients with long-lasting, severe disease (3-5).

So far, we cannot rule out that virus infections contribute to the expansion of CD8⁺CD57⁺ cells, particularly in the patients with long-term immunosuppressive therapy. On the other hand, in none of the patients included in the study there was clinical evidence for virus infections. Moreover, the data derived from the patients with newly diagnosed disease indicate an activation and expansion of T cells, which eventually results in the terminally differentiated CD8⁺ cells, identified by expression of CD57.

So far, the function of CD57 on CD8⁺ cells is not understood; CD57, however, identifies terminally differentiated CD8⁺ cells with the propensity to produce certain cytokines. Whether this cytokine production play a role in WG and MPA, or whether the presence of CD8⁺CD57⁺ merely reflects a history of previous activations within the CD8⁺ T cell compartment cannot be decided as yet. Our data, however, support the hypothesis that CD8⁺ T cells are activated in the context of WG and MPA.

References

- SARRAF P, SNELLER MC: Pathogenesis of Wegener's Granulomatosis: current concepts. *Expert Rev Mol Med* 2005; 13: 1-19.
- HÄNSCH GM, IKING-KONERT C, ANDRASSY K: The pathogenesis of ANCA-associated vasculitides: old hypotheses and new insights. *Clin Nephrol* 2005; 64: 460-4.
- IKING-KONERT C, VOGL T, PRIOR B et al.: T lymphocytes in patients with primary vasculitis: expansion of CD8⁺ T cells with the propensity to activate polymorphonuclear neutrophils *Rheumatology* 2008; 47: 609-16.
- MARINAKI S, NEUMANN I, KÄLSCH AI et al.: Abnormalities of CD4 T cell subpopulations in ANCA-associated Clin Exp Immunol 2005; 140: 181-91.
- VOGT S, IKING-KONERT C, HUG F, ANDRA-SSY K, HANSCH GM: Shortening of telomeres: Evidence for replicative senescence of T cells derived from patients with Wegener's granulomatosis. *Kidney Int* 2003; 63: 2144-51.
- BRENCHLEY JM, KARANDIKAR NJ, BETTS MR *et al.*: Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8⁺ T cells. *Blood* 2003; 101: 2711-20.
- KHAN V, SHARIFF N, COBBOLD M et al.: Cytomegalovirus seropositivity drives the CD8 T cell repertoire toward greater clonality in healthy elderly individuals. J Immunol 2002; 169: 1984-92.

- BANDRES E, MERINO J, VAZQUES B et al.: The increase in IFN-gamma production through ageing correlates with the expanded CD8⁺CD28⁻CD57⁺ subpopulation. Clin Immunol 2000; 96: 230-5.
- WANG EC, LAWSON TM, VEDHARA K, MOSS PA, LEHRER PJ, BORYSIEWICZ LK: The CD8⁺CD57⁺ T cells in patients with rheumatoid arthritis. *Arthritis-Rheum* 1997; 40: 237-48.
- LE PRIOL Y, PUTHIER D, LECUREUIL C et al.: High cytotoxic and specific migratory potencies of senescent CD8⁺CD57⁺ cells in HIVinfected patients and uninfected individuals. *J Immunol* 2006 177: 5145-54.
- JENNETTE JC, FALK RJ, ANDRASSY K et al.: Nomenclature of systemic vasculitis: the proposal of an international consensus conference. Arthritis Rheum 1994; 37:187-92.
- 12. LEAVITT RY, FAUCI AS, BLOCH DA *et al.*: The American College of Rheumatology 1990 Criteria for the classification of Wegener's granulomatosis. *Arthritis Rheum* 1990; 33: 1101-7.
- LUQMANI R, BACON P, MOOTS R et al.: Birmingham vasculitis activity score (BVAS) in systemic necrotizing vasculitis. Q J Med 1994; 87: 671-8.
- DE GROOT K, GROSS WL, HERLYN K, REIN-HOLD-KELLER E: Development and validation of a disease extent index for Wegener's granulomatosis. *Clin Nephrol* 2001; 55: 31-8.
- LAMPRECHT P, GROSS WL: Current knowledge on cellular interactions in the WG-granuloma. *Clin Exp Rheumatol* 2007; 25 (Suppl. 44): S49-51.
- LAMPRECHT P: Off balance: T-cells in antineutrophil cytoplasmic antibody (ANCA)associated vasculitides. *Clin Exp Immunol* 2005; 141: 201-10.
- LAMPRECHT P, MOOSIG F, CSERNOK E et al.: CD28 negative T cells are enriched in granulomatous lesions of the respiratory tract in Wegener's granulomatosis. *Thorax* 2001; 56: 751-7.
- KOMOCSI A, LAMPRECHT P, CSERNOK E et al.: Peripheral blood and granuloma CD4⁽⁺⁾CD28⁽⁻⁾ T cells are a major source of interferon-gamma and tumor necrosis factor-alpha in Wegener's granulomatosis. Am J Pathol 2002; 160: 1717-24.
- VALLEJO AN: CD28 extinction in human T cells: altered functions and the program of T-cell senescence. *Immunol Rev* 2005: 205: 158-69.
- AROSA FA: CD8⁺CD28⁻ T cells: certainties and uncertainties of a prevalent human T-cell subset. *Immunol Cell Biol* 2002; 80: 1-13.
- 21. SCHLESIER M, KASPAR T, GUTFLEISCH J, WOLFF-VORBECK G, PETER HH: Activated CD4⁺ and CD8⁺ T-cell subsets in Wegener's granulomatosis. *Rheumatol Int* 1995: 145: 213-9.
- 22. CAPRARU D, MÜLLER A, CSERNOK E et al.: Expansion of circulating NKG2D⁺ effector memory T-cells and expression of NKG2Dligand MIC in granulomaous lesions in Wegener's granulomatosis. *Clin Immunol* 2008: 127: 144-50.