Expansion of CD8+CD44h Lymphocytes During Growth of SL2 Tumors in DBA/2 Mice

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Article
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Abstract

CD8+ T lymphocytes are critical in the immune response to tumours. Expansion of CD8+ lymphocytes and their accumulation within a tumour is associated with a prolonged survival of cancer patients. CD8+ lymphocyte expansion in tumour-bearing mice can be demonstrated by acquisitions of highly expressed CD44 antigen (CD44h). In this study, we have investigated the levels of CD8+CD44h lymphocytes in peripheral blood during growth of SL2 tumours in DBA/2 mice. The results of our study show that percentages of CD8+CD44h cells in the CD8+ subset were significantly increased in peripheral blood of tumour-bearing DBA/2 mice compared with control mice. However, this expansion of CD8+CD44h lymphocytes does not seem to have antitumour effect, as tumours grow and all tumour-bearing mice die during the second or third week after tumour implantation.

Introduction

CD8+ T lymphocytes are crucial in the adaptive immune response to malignancies and play essential role in inhibiting tumour growth and eradicating cancer cells. Several reports suggest that expansion of CD8+ lymphocytes and their accumulation within a tumour is associated with a prolonged survival of cancer patients [1]. Expansion of human CD8+ lymphocytes is associated with acquisition of CD57 antigen [2]. The predictive and/or prognostic significance of peripheral blood CD8+CD57+ T lymphocytes was shown in patients with advanced renal cell carcinoma [3], melanoma [4], advanced gastric cancer [5] and bladder carcinoma [6]. In some reports CD8+CD57+ T cells are described as highly cytotoxic [7][8][9].

Several lines of evidence indicate that expression of CD57 antigen on human CD8+ lymphocytes is associated with loss of CD28 [10]. In the peripheral lymphoid organs and in the blood of the mouse, all CD4+ and CD8+ T cells express CD28 [11]. Thus, in contrast to human lymphocytes, CD28 loss does not occur in murine T cells. Similarly to CD8+CD57+ in humans, CD8+ lymphocyte expansion in tumour-bearing mice can be demonstrated by acquisition of highly expressed CD44 antigen (CD44h). CD8+CD44h lymphocytes have been regarded as activated / memory T cells [12][13]. Cytotoxic T lymphocytes express high levels of CD44 upon activation [14][15]. CD44 is the principal cell surface receptor for extracellular matrix glycosaminoglycan hyaluronan [16]. Meanwhile, it is known that CD44 is also involved in the extravasation of activated lymphocytes, in lytic effectors functions as well as in T cell activation [17]. Following ligation, CD44 can mediate efficient MHC-unrestricted TCR-independent lysis of virus-infected and tumour cells [18]. Some studies have shown that activated CD8+CD44h lymphocytes play an important role in the lysis of syngeneic tumour target cells in vitro [19]. Dobrzanski et al. [13] have reported that long-term immunity and protection to progressively growing tumour in a murine model is associated with increased numbers of CD8+CD44h cells in both distal (spleen) and local (lung) sites of tumour growth.

In this study, we have investigated the levels of CD8+CD44h lymphocytes in peripheral blood during growth of SL2 tumours in DBA/2 mice.

Materials and methods

Mice and tumours. Female and male DBA/2 mice at the age of 8–12 weeks were obtained from the local breeding facility at the State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania. SL2 lymphoma cells were maintained by weekly intraperitoneal passage in syngeneic DBA/2 mice. SL2 cells were collected from the peritoneal cavity, washed and diluted in RPMI-1640 medium. Solid tumours were induced by subcutaneous injection of 107 SL2 cells in PBS. Each mouse received two injections of SL2 tumour cells on the right and the left flank with the interval of 2 days. Experimental research on animals has been conducted according to recommendations of the Lithuanian Ethics Committee for the Laboratory Animal Use.

FACS analysis of peripheral blood lymphocyte subsets. Two experiments were performed. In the first experiment, peripheral blood was collected from the
tail vein of individual mice (tumour-bearing and control mice) on day 11 after implantation of the first tumour. In the second experiment, peripheral blood was collected from the tail vein of individual mice on day 0 (prior tumour implantation) on day 5 and on day 9 after implantation of the first tumour. Heparin was used as an anticoagulant. Lymphocytes were stained using the following protocol: 20 µl of whole blood of each sample were incubated for 30 min. on ice in the dark with 10 µl of mixture of anti-CD8-FITC/anti-CD44-PE monoclonal antibodies (PharMingen, San Diego, USA). After incubation, red blood cells were lysed by FACS Lysing Solution (Becton Dickinson, San Jose, USA). The samples were analysed on a FACScalibur, (Becton Dickinson, San Jose, USA) flow cytometer with a laser tuned at 488 nm. Data were acquired and analysed with CellQuest software (Becton Dickinson, San Jose, USA). List mode files were collected for 10,000 cells from each sample. Lymphocytes were gated in the forward scatter / side scatter dot plot (R1, Fig. 1A). CD8CD44 dot plot was generated from the gated lymphocytes. CD44 expression on CD8+ T lymphocytes was determined in the CD8/CD44 dot plot, and expression of CD44 antigen was identified as low (R2, Fig.1B), medium (R3, Fig.1B) and high (R4, Fig.1B). The percentage of CD8+CD44h lymphocytes in CD8+ subset was calculated as number CD8+CD44h lymphocytes in all CD8+ lymphocyte subpopulation, divided by 100.

Statistics analysis. Comparisons between the variables of tumour-bearing mice and control mice were performed using the non-parametric Mann–Whitney U test. Differences at p

Results

In the first experiment, tumour-bearing and control mice were killed at day 11 after implantation of the first tumour and peripheral blood from the tail vein was collected. Each group contained 7 mice. Figure 2 shows that percentages of CD8+CD44h lymphocytes in CD8+ subset in peripheral blood of mice with SL2 tumours were significantly higher than in age-matched control mice without tumours.

To answer the question, when and how fast CD8+CD44h lymphocytes expand, a time-course experiment was performed. In this experiment, increase in the levels of CD8+CD44h lymphocytes from day 5 to day 9 were observed. Figure 3 shows the significant increase in CD8+CD44h lymphocyte levels in peripheral blood of tumour-bearing mice on day 9, compared to day 0 and 5.

Thus, growth of SL2 tumours induced expansion of CD8+CD44h lymphocytes in peripheral blood of mice, but these cells could not prevent eventual tumour progression. Tumours grew and all tumour-bearing mice died during the second or third week after tumour implantation. Despite cell-mediated immunity is generated during growth of SL2 tumours in DBA/2 mice, it does not exert a critical role in the inhibition of growth of SL2 tumours.

Discussion

Our results show that CD8+CD44h lymphocyte levels in peripheral blood increase during growth of SL2 tumours in DBA/2 mice. These our results are in concordance with the results of other authors, who have shown that proliferation of CD8+ lymphocytes in mice is most prominent among the CD8+CD44h T cells [20]. Schiavoni et al. [21] observed a long-lasting increase in in vivo lymphocyte proliferation and in the percentage of CD8+CD44h T lymphocytes induced by cyclophosphamide [21].

CD44 (Pgp-1) is a heterogeneous family of molecules with important functions, among which regulation of cell-cell contact and cell matrix adhesion. It is expressed by different haematopoietic cell subpopulations. CD44 is present on the surface of lymphocytes and modulates their homing, allowing their selective binding to specialized endothelium, the high endothelial venules, and to extracellular matrix. It also plays a role in the biology and pathology of cell activation [22].

The expansion of CD8+CD44h lymphocytes in our study does not seem to have antitumour effect, as tumours grow and all tumour-bearing mice die during the second or third week after tumour implantation. These our results seem in contrast with the results of Dobrzanski et al. [13] who showed that elevation in total numbers of CD8+CD44h cell subpopulations was associated with long-term antitumour immunity. It has to be noted, however, that Dobrzanski et al. analysed CD8+CD44h lymphocytes in spleen and lungs of mice, whereas in our study we observed the increase of CD8+CD44h lymphocyte levels in peripheral blood. Our unpublished observations suggest that elevation in levels of CD8+CD44h high lymphocytes in peripheral blood may be accompanied be decrease in numbers of these lymphocytes in the spleen. Thus, distribution of CD8+CD44h T cell subpopulations in tissues and organs of mice might be important for antitumour effect.

Dobrzanski et al. [13] consider CD8+CD44h lymphocytes to be activated and /or memory cells. However, more recent evidence indicates that both
CD8+ central memory cells and CD8+ regulatory T cells express high levels of CD44 [23]. Thus, it makes difficult to distinguish between these two CD8+ cell types based on expression of CD44 antigen. Regulatory functions of CD8+CD44h lymphocytes might impair antitumour immune responses [24].

Finally, a possibility cannot be excluded that following initial clonal expansion, the cells develop activation-induced non-responsiveness, a form of anergy characterized by an inability to produce IL-2. Cells in the state of activation-induced non-responsiveness can carry out effector functions (cytolysis, IFN-gamma production) but cannot continue to proliferate and expand in the face of persisting antigen. Activation-induced non-responsiveness limits the ability of activated cytotoxic T lymphocytes to control tumour growth [25]. Further studies are needed to answer the question why tumours grow and kill the mice despite expansion of the CD8+ lymphocyte compartment.

References


Illustrations

Illustration 1

Figure 1. CD8 CD44h lymphocytes in the peripheral blood of DBA/2 mice.

A. Side scatter (SSC) / forward scatter (FSC) dot plot was used to separate lymphocytes (R1).

B. Expression of CD44 antigen on CD8 T lymphocytes was analysed in the CD8/CD44 dot plot. Cell population with high expression of CD44 antigen on CD8 lymphocytes was identified (R4).
Illustration 2

Figure 2. Percentage of CD8 CD44h lymphocytes in total CD8 T lymphocytes in the peripheral blood of tumour-bearing and control mice. Tumour-bearing and control mice were killed at day 11 after tumour implantation and peripheral blood was collected from tail veins. CD44 expression on CD8 T lymphocytes was analysed in the gated lymphocytes. Expression of CD44 antigen was identified as low (R2, Fig.1B), medium (R3, Fig.1B) and high (R4, Fig.1B). The percentage of CD8 CD44h lymphocytes in CD8 subset was calculated as number CD8 CD44h lymphocytes in all CD8 lymphocytes, divided by 100. Percentage of CD8 CD44h cells in CD8 subset was significantly higher in SL2 tumour-bearing mice compared to control mice (59.33% ± 5.54% vs. 20.97% ± 5.14%, p = 0.0093). Values are given as means ± SD. Each group contained 7 mice.
Illustration 3

Figure 3. Changes in CD8 CD44h lymphocyte levels in the peripheral blood of SL2-bearing mice during tumour growth. Peripheral blood was collected from the tail vein on day 0 (prior to tumour implantation), on 5 and on day 9 after tumour implantation. In this time-course experiment, 8 mice were used. Percentages of CD8 CD44h lymphocytes increased from day 5 to day 9 after tumour implantation.
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