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Vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) provide co-stimulation in positive selection along with survival of selected thymocytes

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Abstract

T-cell differentiation in the thymus depends on positive selection of $CD4^+CD8^+$ double positive (DP) thymocytes by thymic major histocompatibility complex (MHC) molecules. Positive selection allows maturation of only those thymocytes that are capable of self-peptide–MHC recognition. Thymocytes that fail to bind self-peptide-MHC die by apoptosis. An important question in thymocyte differentiation is whether co-stimulation is required for positive selection and on which cells co-stimulatory molecules may be expressed in the thymus. The vascular cell adhesion molecule (VCAM-1) and the intercellular cell adhesion molecule (ICAM-1) are known to be potent co-stimulatory molecules in activation of peripheral T-cells by interacting with the integrins VLA-4 and LFA-1, respectively. We were prompted to investigate whether VCAM-1 and ICAM-1 may also act as co-stimulators during selection of thymocytes. By using recombinant proteins of murine VCAM-1 and ICAM-1 fused to the Fc region of human IgG1 (rVCAM-1, rICAM-1) we examined the capacity of VCAM-1 and ICAM-1 to act as co-stimulatory molecules in positive selection in vitro. Triggering the CD3/TCR complex together with co-stimulation applied by rVCAM-1 or rICAM-1 induced the generation of $CD4^+$ single positive (SP) thymocytes from $CD4^+CD8^+$ DP thymocytes whereas either signal alone did not result in generation of $CD4^+$ SP thymocytes. VCAM-1 and ICAM-1 act therefore as co-stimulatory molecules in thymocyte positive selection in vitro. The generation of $CD4^+$ SP cells is accompanied by cell survival both when it was co-stimulated with rVCAM-1 and with rICAM-1. Importantly we show here that VCAM-1 expression in the murine thymus is restricted to cortical F4/80 positive hematopoietic antigen presenting cells (hAPC) present exclusively in the cortex whereas expression of ICAM-1 has been reported on the epithelium both in cortex and medulla. This suggests that not only the cortical epithelium may use the co-stimulatory molecule ICAM-1 to mediate positive selection, but also cortical hAPCs may contribute to positive selection of thymocytes by using the co-stimulator VCAM-1.

Keywords: Thymus; Thymocyte; Cortex; Positive selection; T-cell selection; T-cell differentiation; MHC; TCR; Hematopoietic antigen presenting cell; ICAM-1; VCAM-1; CD3; Co-stimulation; Co-stimulatory molecule; F4/80

1. Introduction

T lymphocytes make use of the CD3-T-cell receptor (TCR) complex during specific recognition of antigen in the context of products of self-major histocompatibility complex (MHC) genes expressed on the surface of antigen-presenting cells (APC). The CD3–TCR complex participates in signal transduction to initiate the activation of T-cells (Weiss, 1990; Springer, 1990). In addition, the activation of T-cells requires the participation of co-stimulatory signals, which are provided by co-stimulatory molecules present on APCs (Damle and Aruffo, 1991; Linsley et al., 1991; Damle et al., 1992; van Seventer et al., 1991).

During T-cell development positive and negative selection require likewise the interaction of the TCR molecules expressed on developing thymocytes with MHC/peptide complexes on the surface of thymic antigen presenting cells. The rescue of $CD4^+CD8^+$ DP thymocytes in positive selection with self-MHC-restricted TCR is accompanied by lineage deci-

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sion: Thymocytes selected by recognition of MHC class I molecules down-regulate CD4 and become CTL precursors maintaining the co-receptor CD8 (Sha et al., 1988; Kisielow et al., 1988), whereas those selected by MHC class II down-regulate CD8 and become Th cell precursors maintaining the co-receptor CD4 (Kaye et al., 1989; Berg et al., 1989). It has been shown that negative selection of $CD4^+CD8^+$ DP thymocytes requires both a TCR signal and co-stimulation to induce apoptosis (Punt et al., 1994). Moreover it has been proposed that for positive selection of thymocytes co-stimulation may be required as well (Cibotti et al., 1997; Ohoka et al., 1996). Different molecules expressed on the surface of $CD4^+CD8^+$ DP thymocytes have been shown to possess "coinducing" activity when activated in vitro by stimulation with immobilized antibodies directed against these molecules together with co-immobilized antibodies against the TCR (Cibotti et al., 1997).

It is well-established that the adhesion receptors VCAM-1 and ICAM-1 co-stimulate T-cell proliferation via the integrins VLA-4 and LFA-1, respectively (Damle and Aruffo, 1991; Damle et al., 1992; van Seventer et al., 1991). Therefore we were prompted to investigate whether VCAM-1 and ICAM-1 may also co-stimulate positive selection of thymocytes. Indeed we report here that when $CD4^+CD8^+$ DP thymocytes are cultured in the presence of plate bound antibodies directed against TCR/CD3 co-immobilized with rVCAM-1 or rICAM-1 in vitro positive selection is induced resulting in generation of CD4 SP cells. Positive selection of CD4 SP cells is accompanied by survival of thymocytes. Interestingly we observed that in the murine thymus VCAM-1 is almost exclusively expressed on $F4/80^+$ macrophages in the cortex whereas it is known that ICAM-1 is expressed on the thymic epithelium in cortex and medulla. The expression of ICAM-1 on the epithelium is in line with a function of cortical epithelial antigen presenting cells in positive selection. Interestingly, however, the fact that the co-stimulatory molecule VCAM-1 is expressed exclusively on cortical hAPC suggests that these hematopoietic cells may contribute to thymic positive selection as well.

2. Materials and methods

2.1. Reagents

H57-597 (anti-TCRα/β, Biolegend), anti-CD8-APC (Pharmingen), anit-CD4-PERCP (Pharmingen), anti F4/80 (Pharmingen), anti-VCAM-1-biotin (MVCAM.A; Biolegend), anti-CD69-PE (eBioscience), Annexin-V, 7-AAD (Pharmingen), recombinant VCAM-1-Fc and ICAM-1-Fc (R&D Systems), Human IgG1 isotype control antibody (Serotec).

2.2. Cell preparations

Thymi were obtained from up to 2 weeks old C57BL/6 mice and after mincing the thymus a single cell suspension was prepared. Fragments of some specimens were flash-frozen in liquid nitrogen and stored at −80 °C.

2.3. In vitro positive selection

$CD4^+CD8^+$ DP thymocytes were isolated from total thymocyte suspensions using MACS CD8 T-cell isolation kit (Miltenyi). 96-well flat bottom tissue culture plates (Maxisorp, NUNC) were pre-coated with anti-CD3 antibodies (clone 145-2C11 at 10, 100 and 1000 ng/ml) and/or recombinant VCAM-1 or ICAM-1 (at 1, 2 and 5 μg/ml, diluted in 0.05 M sodium hydrogen carbonate buffer, pH 9.2) and incubated for 1 h at 37 °C. Wells were washed in RPMI containing 10% FCS. Thymocytes were added to the wells in RPMI containing 10% FCS in the presence or the absence of the specific MEK inhibitor U0126 (10 μM, Calbiochem). Cells were harvested after 65 h of culture and subjected to flow cytometry by staining with CD69-PE, CD4-PERCP, CD8-APC. Viable cells were clearly distinguishable from apoptotic cells according to forward side-scatter criteria, which was confirmed by the apoptosis markers Annexin-V (BD Pharmingen) and 7-AAD (Molecular Probes, Eugene, OR) (data not shown).

2.4. Immunohistochemical analysis

Briefly, cryostat sections of thymic tissue were cut in 8 μm sections, air-dried overnight, and fixed in acetone for 10 min at room temperature. Primary antibodies diluted in PBS/1% BSA were added directly onto the sections and incubated for 30 min. For immunofluorescence, Alexa Fluor® 488 $F(ab')_2$ fragment of goat anti-rat IgG and Alexa Fluor® 594 conjugated Avidin (both Molecular Probes, Eugene, OR) were used. As primary antibodies F4/80 and VCAM-1 were used. A sequential staining procedure was performed. First sections were incubated with anti-F4/80. After washing secondary goat ant-rat Alexa 488 was added. After washing again, sections were blocked with rat serum followed by incubation of biotinylated anti-VCAM-1 antibodies. Slides were washed again and incubated with Alexa 594-conjugated Avidin.

3. Results

3.1. Costimulatory effect of VCAM-1 and ICAM-1 co-immobilized with anti-CD3 antibodies induces in vitro positive selection

Positive selection of thymocytes occurs at the $CD4^+CD8^+$ DP stage of T-cell development. Therefore $CD4^+CD8^+$ DP thymocytes were isolated for in vitro positive selection (Fig. 1). At varying concentrations rVCAM-1, rICAM-1 (1, 2 and 5 μg/ml) and anti-CD3 mAb (1, 10, 100, 1000 ng/ml) were immobilized alone or co-immobilized in microtiter plates. Thymocytes were cultured on these plates for 65 h and subsequently analysed by FACS for their expression of CD4 and CD8. Apoptotic cells in the cultures were distinguished from viable cells by Forward Scatter. This approach was confirmed by using the apoptosis markers Annexin V and 7-AAD (data not shown). We observed that CD4 SP thymocytes develop from $CD4^+CD8^+$ DP thymocytes when anti-CD3 mAb is co-immobilized with either rVCAM-1 or rICAM-1. Fig. 2 demonstrates a typical