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Activation-induced cell death signalling in CD4+ T cells by staphylococcal enterotoxin A

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Abstract

Staphylococcal enterotoxin A (SEA) is a potent stimulator of CD4+ and CD8+ T cells, the immunotoxic action of which remains unclear. We investigated the *in vitro* effects of SEA on freshly isolated human peripheral blood lymphocytes depleted of CD8+ T cells. Proliferation and flow cytometry analysis indicated that SEA generated an activation-induced cell death (AICD) phenomenon that was characterized by an increased expression of the chemokine receptor CCR5 on the CD4+/CD45RO+ T cell subset. Incubation of cells with glycoprotein secretion inhibitor monensin A completely blocked cell proliferation, affecting mainly the CD4+/CD45RO+ T cell subset. The IL-2 mRNA levels were increased just hours after SEA stimulation, accompanied by an increase in the expression of CD25, indicating a possible involvement of IL-2 in the AICD process. We observed a 15-fold mRNA reduction of the transcription factor Yin Yang 1 (YY1) at the proliferation peak, and an increase of the receptors CCR5, CD95 and DR5 on the CD45RO+/CD4+ T cell subset. These findings suggest that SEA triggers a TCR-mediated AICD mechanism in CD4+ T cells, the intracellular signalling of which is probably modulated, at least, by YY1.

Keywords: Staphylococcal enterotoxin A; CD4+ T cells; YY1; Activation-induced cell death

1. Introduction

Staphylococcal enterotoxin A (SEA) belongs to a large family of enterotoxins produced by *Staphylococcus aureus* that have been implicated in food poisoning and allergic reactions (Johnson et al., 1991; Balaban and Rasooly, 2000). Staphylococcal toxins are the second most common cause of food-borne illnesses in the US, SEA being responsible for the majority of those cases (Levine et al., 1991; Bunning et al., 1997). Even small amounts of SEA in the food are sufficient to cause intoxication as evidenced from an outbreak in the US, the mean amount of which was found in the milk to be only 0.5 ng/ml (Evenson et al., 1988). It is very difficult to eliminate SEA from contaminated food because it is a relative small molecule, resistant to inactivation by gastrointestinal proteases and very stable to heat (Denny et al., 1971; Hernandez et al., 1993).

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The immunotoxicity of staphylococcal enterotoxins is attributed to their ability to activate CD4+ and CD8+ T cells to an antigen-independent proliferation through an alternative antigen presentation process (White et al., 1989). Unlike conventional antigens, SEA acts as a bridge to the class II major histocompatibility complex (MHC) outside the peptide binding groove on antigen presenting cells (APC), and the variable region of the TCR β-chain on the CD4+ T cells. The number of major Vβ regions of TCR in the human T cell repertoire is approximately 24 (Herrmann et al., 1989; Champagne et al., 1993). SEA can bind to more than one Vβ region, potentially capable of activating up to 25% of T cells (Hudson et al., 1993; Li et al., 1999). The activation signal of SEA is amplified by the interaction of co-stimulatory molecules that are present in conventional antigen presentation (Malissen, 2003). The release of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and interleukin 2 (IL-2), drives the immune system towards a Th1 proinflammatory response (Norrby-Teglund et al., 1994; Llewelyn and Cohen, 2002) that results in generalized enhanced activation of T cells (Wang et al., 1997; Gorak-Stolinska et al., 2002). The subsequent apoptosis of these cells is often referred to as activation-induced cell death (AICD).

Although the general interaction of SEA with immune cells is well studied, and the structure of SEA has been deciphered by X-ray crystallography (Schad et al., 1995) (Cuff et al., 2003), the signals that drive T cells to AICD remain unclear (Gerwien et al., 1999; Hu et al., 2005). Here, we investigated the role of IL-2 and its receptor CD25 on the SEA-activated T cells, as well as the involvement of the transcription factor YY1, which has been reported to act as a negative regulator of CD95L- and TRAIL-mediated cell death via transcriptional repression of the receptors CD95 and DR5 (Garban and Bonavida, 2001). We evaluated these parameters in primary human CD4+ T cells and in the CD4+/CD45RO+/CCR5+ subpopulation which represents the effector/memory arm of the CD4+ T cell immune system.

2. Materials and methods

2.1. Cell isolation

Buffy coats from healthy, HIV-1/hepatitis b sera-negative blood donors were obtained from Venizelio Hospital Blood Transfusion Service, Heraklion, Crete. Peripheral blood mononuclear cells (PBMCs) were isolated using ficoll-paque (Amersham–Pharmacia, Uppsala, Sweden) according to the manufacturer's protocol and were cultured in RPMI-1640 medium supplemented with antibiotics and 5% human serum (Zafiropoulos et al., 1997). The depletion of CD8+ T cells was carried out with the MACS system (Mylteniil Biotech, Germany) using anti-PE microbeads and anti-CD8 PE, according to the manufacturer's protocol.

2.2. Proliferation assay

For the proliferation assays, 10^6 PBMCs per ml were cultured in 96-well plates (Costar) in RPMI-1640, containing 5% human serum, and 1 ng/ml staphylococcal enterotoxin A (SEA) (Sigma–Aldrich Chemical Co.). Cell cultures in triplicate wells were incubated at 37 °C in 5% CO_2 in a humidified incubator for 1, 3, 5, 7, 9 and 11 days. Cells were harvested after a pulse period of 18 h with ^{3}H-thymidine (Amersham, UK) and proliferation was measured as counts per minute in a LS1701 beta counter (Beckman, USA). Finally, when appropriate, cells were treated with 10 mM Monensin (Calbiochem) for 30 min and then cultured as described above.

2.3. Phenotypic analysis of T cells by flow cytometry

The phenotypic analysis of T cells was carried out by flow cytometry. Briefly, 10^6 PBMCs per ml were cultured in 24-well plates (Costar) in RPMI-1640, containing 5% human serum and 1 ng/ml SEA. Cells were centrifuged at 1300 rpm for 10 min at 25 °C and were resuspended in 100 μl cold PBS supplemented with 2% inactivated foetal calf serum (GIBCO), pH 7.4. They were incubated for 20 min on ice with 20 μl fluorochrome-labelled antibodies and after two washes with cold PBS, the pellet was resuspended in 500 μl PBS and the cells were analysed immediately by FACS (Callibur, Becton–Dickinson, USA) using the CELLQuest programme. For the analysis of the chemokine receptors, CCR5 and CXCR4, on CD4+ CD45RO+ T cells, measurements were taken at the day of maximum proliferation. For the kinetic analysis of the expression of CD95 and CD25 on CD4+ CD45RO+ T cells, measurements were taken after 1, 3, 5, 7, 9 and 11 days. The fluorochrome-labelled antibodies were CD45RO− FITC (Mouse IgG2a,k, UCHL1), CD45RO− PE (Mouse IgG2a,k, UCHL1), CXCR4− PE (Mouse IgG2a,k, 12G5), CCR5− PE (Mouse IgG2a,k, 2D7/CCR5), CD4 PE-Cy5 (Mouse IgG1,k, RPA-T4) CD95− PE (Mouse IgG1k, DX2) and CD25 (Mouse IgG1k, M-A251) from BD Pharmingen. For the DR5 expression analysis cells were incubated with 1 μl anti-DR5 (mouse polyclonal anti-human DR5, Biosource) for 1 h at room temperature and then incubated for 30 min with secondary antibody (*R*-phycoerythrin-conjugated goat anti-mouse IgG, Jackson immunoresearch).

2.4. Measurements of cell apoptosis

The apoptotic effect of SEA was analysed by flow cytometry. Briefly, 1×10^6 cells were collected at preferred time