

Superantigen-Induced T Cell Death by Apoptosis: Analysis on a Single Cell Level and Effect of IFN- γ and IL-4 Treatment

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Key Words

Apoptosis · Superantigen · T cell · IFN- γ · IL-4

Abstract

Background: A role of bacterial superantigens in several chronic inflammatory diseases has previously been proposed. Many of these diseases are associated with an imbalance of the T helper cell subsets and their cytokine production. **Methods:** Peripheral blood mononuclear cells from healthy donors were incubated with various concentrations of staphylococcal enterotoxin B (SEB) with or without IL-4 or IFN- γ . After different time points cell activation, proliferation, Fas expression, cytokine release and cell death via apoptosis were detected. **Results:** SEB treatment resulted in sequential T cell activation, proliferation, Fas expression, cytokine release, subsequently followed by cell death via apoptosis in a dose- and time-dependent manner. This biphasic effect occurred preferentially in SEB-responsive cells represented by the expression of V β 3 and V β 12 on T cells. A strong relationship between T cell activation and apoptosis was observed. The amplitude between these events increased with the dose of SEB. The highest rate of apoptotic T cells was observed at a dose of 1,000 ng/ml SEB. Addition of IFN- γ to SEB-treated cells significantly re-

duced the rate of apoptotic cells, whereas IL-4 prevented apoptosis only in SEB-untreated cells. **Conclusion:** These results support the concept that the dose of superantigen exposure determines the rate of T cell proliferation and subsequent cell death. This T cell immune response is modulated by the presence and the type of cytokines.

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Introduction

Exotoxins produced by certain strains of *Staphylococcus aureus* act as superantigens, they are highly potent immunostimulatory molecules and have dramatic effects on T cells. They can strongly activate T lymphocytes in an oligoclonal fashion through interaction with the V β region of the TCR [1–3]. For many superantigens the responsive TCR-V β elements have been mapped. For example, the superantigen Staphylococcus enterotoxin B (SEB) reacts with V β 3 and V β 12, but not with V β 5- and V β 8-expressing T cells [4–6]. After in vivo administration of superantigens in mice, specific V β -expressing T cells are rapidly activated to proliferate and release cytokines. This initial expansion is followed by deletion of responsive T cells via apoptosis [7–11]. Apoptotic, SEB-responsive murine T cells express high levels of Fas and Fas ligand, implicating

the Fas pathway in superantigen-mediated apoptosis in vivo [12]. In vitro models of apoptotic cell death show that cytokines can either inhibit or augment apoptotic cell death depending on the investigated cell system and on the stimulus used to induce apoptosis [13–17].

Superantigens have been associated with acute, life-threatening illnesses including toxic shock syndrome, gram-positive sepsis and Kawasaki's disease as well as with chronic inflammatory conditions including psoriasis, arthritis or atopic dermatitis (AD) [reviewed in 18]. Many of these conditions are associated with an imbalance of the T helper cell subsets Th1 and Th2, e.g. a predominance of the Th2 over Th1 cell response has been reported in AIDS, sepsis and allergic diseases. In contrast, a predominance of Th1 over Th2 has been shown in rheumatoid arthritis, contact hypersensitivity, 'Crohn's disease' and in several autoimmune disorders [19].

In AD, for example, the impact of *S. aureus*-derived exotoxins on disease exacerbation has been documented [20, 21]. Further studies have shown that chronicity of inflammation is accompanied by a shift from lesional Th2 towards Th1 cytokine production [21, 22]. In addition successful therapy of AD led to induction of apoptosis in intradermal T helper cells and to a downregulated lesional expression of IFN- γ [22–25].

These observations suggest that superantigen exposure may modulate the state of T cell activity. It was the aim of this study to examine the relationship between superantigen-induced T cell activation and proliferation and subsequent development of T cell apoptosis. It was of particular interest to examine whether the balance between proliferation and apoptosis varied between low and high doses of superantigen. This study was carried out in an in vitro culture system with lymphocytes from normal healthy individuals utilizing SEB as a model superantigen.

Materials and Methods

Cell Preparation and Cell Culture Conditions

PBMC were obtained by lymphoprep gradient centrifugation [Life Technologies, Gaithersburg, Md., 20 min, 2,000 rpm, room temperature (RT)] of heparinized whole blood drawn from healthy adult individuals (range of V β 3 + V β 12-positive T cells between 2.7 and 9.6%, range of V β 5 + V β 8-positive T cells between 5.1 and 14.7%). Isolated cells from the interphase were washed 3 times in PBS, adjusted to a concentration of 2×10^6 cells/ml and cultured in RPMI 1640 (Biochrom, Berlin, Germany) supplemented with 10% heat-inactivated FCS (Behringwerke, Marburg, Germany), 10 IU/ml penicillin, 100 μ g/ml streptomycin (Biochrom), 0.2 μ g/ml amphotericin B (Life Technologies), and 2 μ M glutamine (Biochrom) at 37 °C

in a humidified atmosphere of 5% CO₂ and 95% air. For the detection of apoptosis, analysis of cell surface marker expression and measurement of cytokine production 2×10^6 cells were cultured in 24-well flat-bottom tissue culture plates (Falcon, Heidelberg, Germany). PBMC were cultured with medium alone or with 400 U/ml IL-4, 2,000 U/ml IFN- γ (Thomae, Biberach, Germany), or 2 μ g/ml anti-IFN- γ (Boehringer Mannheim, Germany) for 24 h prior to a washing step and the addition of SEB (Toxin Technologies, Fla.) treatment.

Cell Proliferation Assay

Triplicates of 4×10^5 cells were incubated in 96-well round-bottom microtiter plates (Greiner, Nürtingen, Germany) for 3 days. For the last 24 h of culture 1 μ Ci [³H]TdR/well was added. Cells were harvested and counts were measured in a liquid scintillation counter (Beckmann, Munich, Germany).

Flow Cytometry

10,000 cells were analyzed using a Calibur flow cytometer (Becton-Dickinson) equipped with a single 488-nm argon ion laser interfaced with a Macintosh Quadra 650 computer. The green (FITC) fluorescence was collected through a 530/30-nm band-pass filter, red [Phycoerythrin (PE) and propidium iodide (PI)] fluorescence through a 582/42-nm band-pass filter and the red fluorescence for PerCP and 7-amino-actinomycin D (7-AAD) through a 650 nm long pass filter. Cell debris was excluded by a combination of forward light scatter (FSC) and 90° scatter (SSC). For the analysis of cell surface marker expression cells were collected after culture and washed twice with PBS (10 min, 2,000 rpm, RT). 2.5×10^5 cells were incubated with saturating concentrations of Ab for 15 min at 4 °C in the dark, washed once in staining buffer, and analyzed by flow cytometry using a gate for lymphocytes. The following Ab combinations were used: IgG1-FITC/IgG2a-PE (control), CD45-FITC/CD14-PE (Leukogate), Fas-FITC/CD3-PE/CD4-PerCP (Becton-Dickinson), V β 3 + V β 12-FITC (Immunotech, Marseille, France)/CD25-PE (Becton-Dickinson)/CD4-PerCP, V β 5 + V β 8-FITC (Immunotech)/CD25-PE/CD4-PerCP, and V β + V β 12-FITC/Fas-PE.

PI Staining [26]

Cells were washed with PBS and fixed in 70% ice-cold ethanol for 60 min at 4 °C. After washing in PBS cells were resuspended in 0.5 ml PBS containing 1 mg/ml RNase (Sigma Chemical Co., Germany; type 1-A, boiled for 15 min at 100 °C to inactivate DNase) and incubated for 15 min at RT. Thereafter 0.5 ml PI solution (Sigma; 100 μ g/ml PBS) was added for 15 min at RT in the dark. Cells were washed once in PBS and kept at 4 °C in the dark until measurement.

TUNEL Assay [27, 28]

Cells were harvested, washed and fixed in 1% paraformaldehyde (Fluka, Buchs, Switzerland) in PBS (pH 7.4) for 15 min at 4 °C. After washing twice in PBS, they were resuspended in 70% ice-cold ethanol for 60 min in the dark at 4 °C. Cells were washed twice in PBS and resuspended in 50 μ l cacodylate buffer (Boehringer Mannheim) containing 0.2 M potassium cacodylate, 25 mM Tris-HCl (pH 6.6), 2.5 mM cobalt chloride, 0.25 mg/ml BSA, 100 U/ml terminal deoxynucleotidyltransferase (Boehringer Mannheim) and 0.5 nM biotin-16-dUTP (Boehringer Mannheim). Cells were incubated for 30 min at 37 °C, rinsed in PBS and resuspended in 100 μ l saline-citrate buffer (Sigma) containing 2.5 μ g/ml fluoresceinated avidin (PharMingen, Hamburg, Germany), 0.1% Triton X-100 (Sigma) and 5% (w/v) BSA

(Sigma). After incubation for 30 min at RT in the dark cells were washed twice in PBS containing 0.1% Triton X-100 and again in PBS alone and were kept in the dark at 4°C until measurement. For the simultaneous detection of Vβ-expressing T cells and apoptosis cells were stained with a combination of Vβ3-plus Vβ12-FITC-labelled Ab before fixation and streptavidin-PE (PharMingen) was used to detect biotin-16-dUTP.

7-AAD Staining

Cells were harvested, washed and resuspended in 20 µg/ml 7-AAD (Sigma) in PBS [29, 30], incubated at 4°C in the dark for 20 min followed by immediate analysis on the flow cytometer.

PI Exclusion Assay

After the staining of cell surface antigens with mAb cells were incubated with 5 µg/ml PI in PBS for 5 min at RT. Cells were washed, resuspended in PBS and immediately analyzed on the flow cytometer. Nonviable cells appeared as red fluorescing cells.

Measurement of Cytokines in Cell Culture Supernatants

After 48 and 72 h cell-free culture supernatants were harvested and analyzed for IL-5 and IFN-γ production by ELISA. 96-well ELISA plates (Nunc) were coated with either monoclonal mouse anti-human IL-5 (PharMingen, 2 µg/ml), or anti-human IFN-γ Ab (Medgenix Diagnostics, Ratingen, Germany; 2.5 µg/ml) diluted in PBS. After overnight incubation at 4°C, plates were washed twice with PBS/Tween and blocked with 3% BSA (Sigma)/PBS buffer (w/v) for 1 h at RT. After washing, wells were incubated with either cytokine standards or tissue culture supernatants in duplicates for 4 h at RT. The following biotin-labelled secondary mAb were incubated at a concentration of 1 µg/ml for 45 min at RT: rat anti-human IL-5 (PharMingen) and anti-human IFN-γ (Medgenix Diagnostics). After washing, plates were incubated with streptavidin-peroxidase (Calbiochem, Germany) diluted in 3% BSA/PBS buffer for 30 min at RT. The substrate for the reaction was tetramethylbenzidine (Fluka) diluted 1:100 in Gallati buffer. The reaction was stopped with 4 N sulphuric acid and plates were measured at 450/490 nm. The sensitivities of these assays were for IL-5 30 pg/ml and for IFN-γ 4 pg/ml.

Statistical Analysis

Statistical analysis was performed by the Wilcoxon matched-pair signed-rank test.

Results

Stimulation of T Cells with SEB Leads to Activation, Proliferation, Fas Expression and Cytokine Secretion

Stimulation of PBMC with SEB resulted in marked T cell activation. As shown in figure 1a, SEB induced proliferation in a dose-dependent manner. These findings were paralleled by the dose-dependent expression of IL-2R α chain (CD25) on PBMC with plateau levels at 48 h. The cytokines IL-4 and IFN-γ had no effect on CD25 expression, but slightly enhanced (not statistically significant) the degree of T cell proliferation due to stimulation with 0.001–1,000 ng/ml SEB. Less than 2% of untreated cells

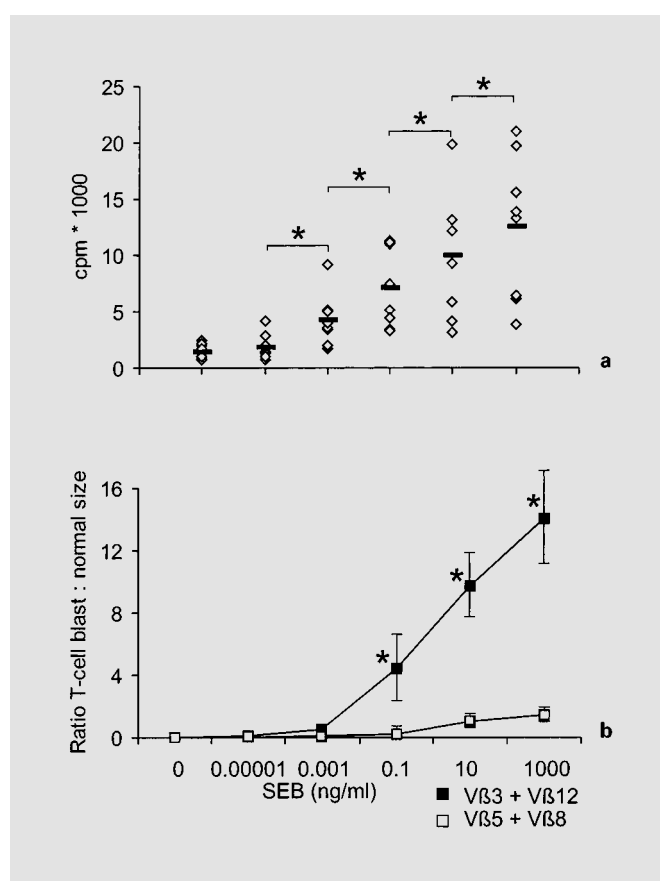


Fig. 1. Dose dependency of SEB-induced proliferation (a) and blast formation on SEB-responsive and nonresponsive T cells (b). Cells were cultured with SEB in concentrations indicated for 48 h (blast formation) or 72 h (proliferation). a Results from 8 independently performed experiments and their means are shown. b Data represent the arithmetic means $\pm$ SD of 8 experiments. For each SEB dose the following ratio was calculated: percentage of T cell blasts to non-blasts. Significant differences: * $p < 0.05$.

expressed Fas on day 0, but the Fas expression increased strongly with increasing doses of SEB. Fas upregulation was highest on day 2 and declined with time. Whereas culture of PBMC with 0.00001 ng/ml SEB nearly had no effect, 0.1–1,000 ng/ml SEB significantly increased proliferation, CD25 and Fas surface expression in a dose-dependent manner.

IFN-γ and IL-5 were measured as representative Th1 and Th2 cytokines. Kinetic experiment revealed that upon SEB stimulation IFN-γ secretion peaked after 48 h, whereas IL-5 secretion was highest after 72 h. As shown in table 1, stimulation of PBMCs with SEB resulted in production of IFN-γ and IL-5, whereas unstimulated cells produced minimal amounts of IFN-γ and no detectable

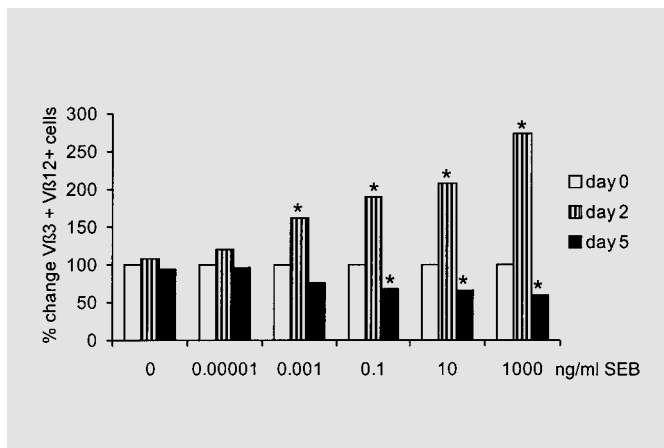


Fig. 2. Effect of SEB stimulation on the distribution of SEB-responsive cells (Vβ3 and Vβ12). PBMC were cultured for 2 and 5 days with SEB as indicated. The frequency of Vβ3- and Vβ12-expressing T cells on day 0 was calculated as 100% and the effect of SEB over time was compared to these values. Shown are the arithmetic means of 8 experiments. Significant differences: * $p < 0.05$ vs. day 0.

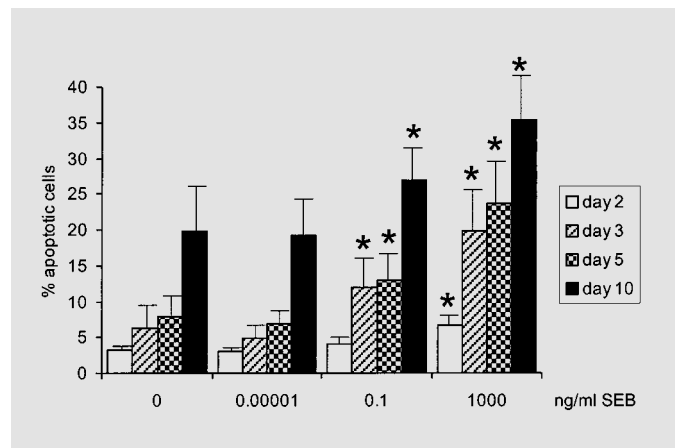


Fig. 3. Dose- and time-dependent induction of apoptosis by SEB. PBMC were cultured for different time intervals in the presence of SEB as indicated. Cells were harvested and DNA was stained by PI as described in Materials and Methods. The arithmetic means $\pm$ SD of 8 experiments are shown. Significant differences between two SEB concentrations: * $p < 0.05$ (0.00001 vs. 0.1 or 0.1 vs. 1,000 ng/ml SEB).

Table 1. Effect of SEB on cytokine production

SEB treatment, ng/ml	IL-5, pg/ml	IFN- γ , ng/ml
0	<30	0.30
0.00001	<30	0.25
0.001	130	4.80
0.1	200	48.70
10	260	73.50
1,000	270	90.00

2×10^6 PBMC/ml were cultured in medium alone or with SEB in concentrations as indicated. ELISA assays were performed on supernatants collected on day 2 for measurement of IFN- γ and on day 3 for IL-5.

levels of IL-5. Treatment of PBMC with IFN- γ augmented the production of IFN- γ , and treatment with IL-4 augmented IL-5 production (data not shown).

Identification of the SEB-Responsive T Cell Subsets

Activated T cells are known to increase in volume, which can be detected by flow cytometry using FSC analysis. Thus, FSC analysis provides a convenient tool to differentiate between activated ('T cell blasts') and resting T cells. TCR-Vβ3- and Vβ12-expressing cells (representing SEB-responsive cells) increased in size upon SEB stimulation, whereas TCR-Vβ5 and Vβ8 T cells (representing

SEB nonresponsive cells) did not show blast formation. Moreover, the upregulation of CD25 occurred predominantly in the SEB-responsive subsets. Blast formation (fig. 1b) and CD25 expression were strongly induced by 0.1–1,000 ng/ml SEB in SEB responsive T cells, but only marginally in SEB-nonresponsive T-cells.

SEB Sequentially Triggers Expansion and Deletion of T Cells

Upon SEB stimulation the percentage of the SEB-responsive T cell subsets Vβ3 and Vβ12 increased significantly within the first few days in a dose-dependent manner. As shown in figure 2, stimulation of PBMC in vitro with 1,000 ng/ml SEB resulted in a nearly 3-fold relative increase in this subset. Following the initial clonal expansion, the proportion of SEB-responsive T cells significantly decreased by day 5 and was even lower than at the beginning of culture (SEB ≥ 0.1 ng/ml).

Induction of Apoptosis by SEB Is Dose- and Time-Dependent

The frequency of apoptotic cells was measured by PI staining of DNA at different time points. As shown in figure 3, the percentage of apoptotic cells increased dose-dependently between SEB concentrations of 0.1 and 1,000 ng/ml. The increase of apoptotic cells was detectable as early as on day 3 and further increased until day 10. To confirm the apoptotic mode of cell death the

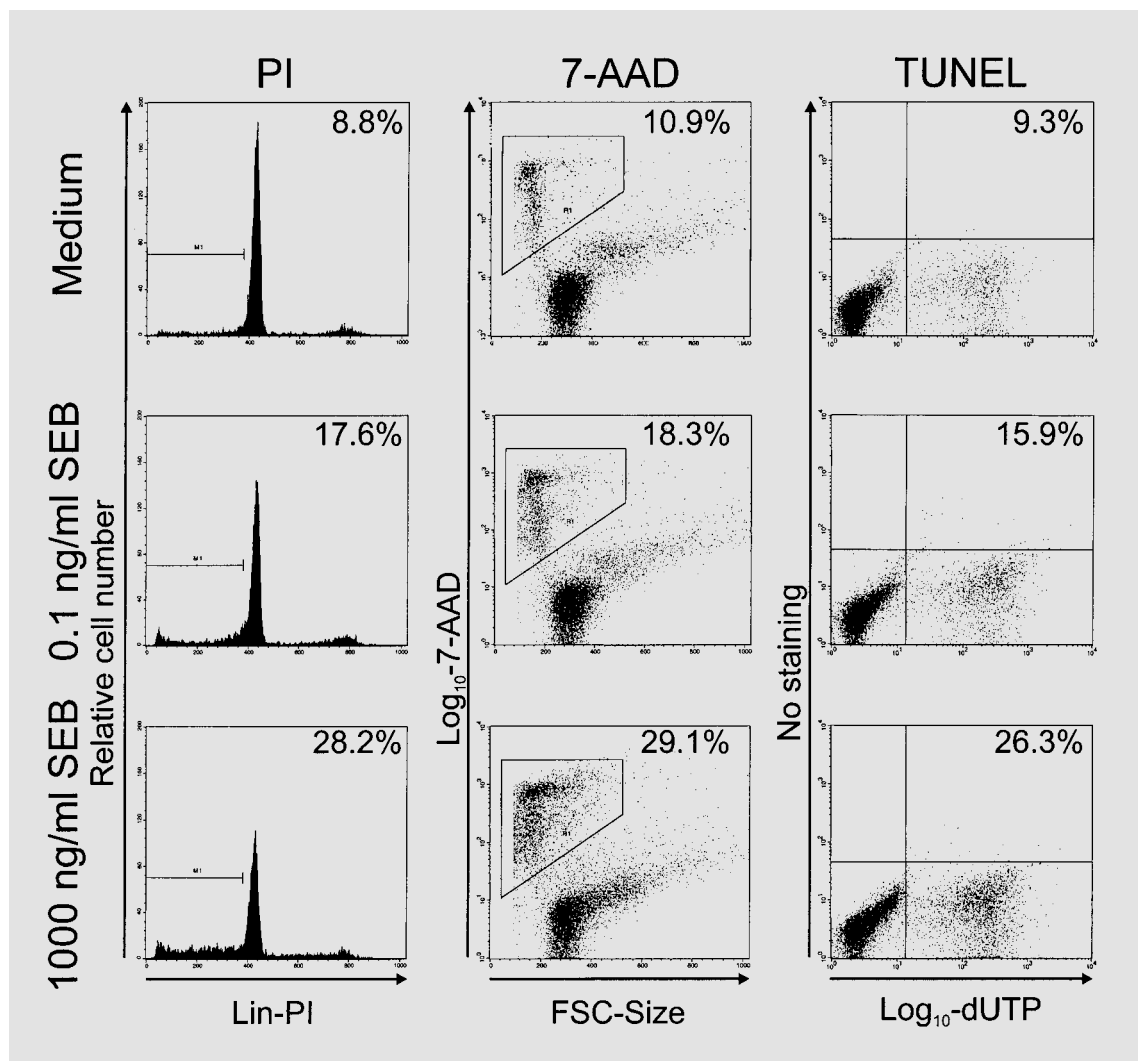


Fig. 4. Assessment of apoptosis by PI staining assay, 7-AAD method and TUNEL technique. PBMC were cultured for 5 days with medium alone, 0.1 or 1,000 ng/ml SEB as indicated. The percentage of apoptotic cells is indicated. All assays were performed as described in Materials and Methods.

7-AAD assay and TUNEL were performed and the data confirmed the results obtained with the PI assay (fig. 4). Double staining of V β 3- and V β 12-positive cells with Fas, PI or dUTP revealed that nearly all of the V β 3- and V β 12-expressing cells showed Fas expression and signs of apoptotic cell death (fig. 5).

IFN- γ and IL-4 Differentially Modulate SEB-Driven Cell Death

To analyze the effects of IFN- γ and IL-4 on the regulation of SEB-induced apoptosis, IL-4 or IFN- γ were added to SEB-stimulated or nonstimulated cells. In unstimulat-

ed cells the addition of IL-4 or IFN- γ prevented apoptosis to a large extent ($p < 0.05$). In this regard IL-4 had a stronger effect than IFN- γ . In SEB-stimulated cells, the addition of IL-4 did not have an effect on apoptosis, whereas IFN- γ significantly ($p < 0.05$) reduced the frequency of apoptotic cells (fig. 6). However, treatment with IL-4 and IFN- γ had a differential effect on the induction of Fas expression. As shown in table 2, treatment with IL-4 and with IFN- γ increased the frequency of Fas-expressing T cells.

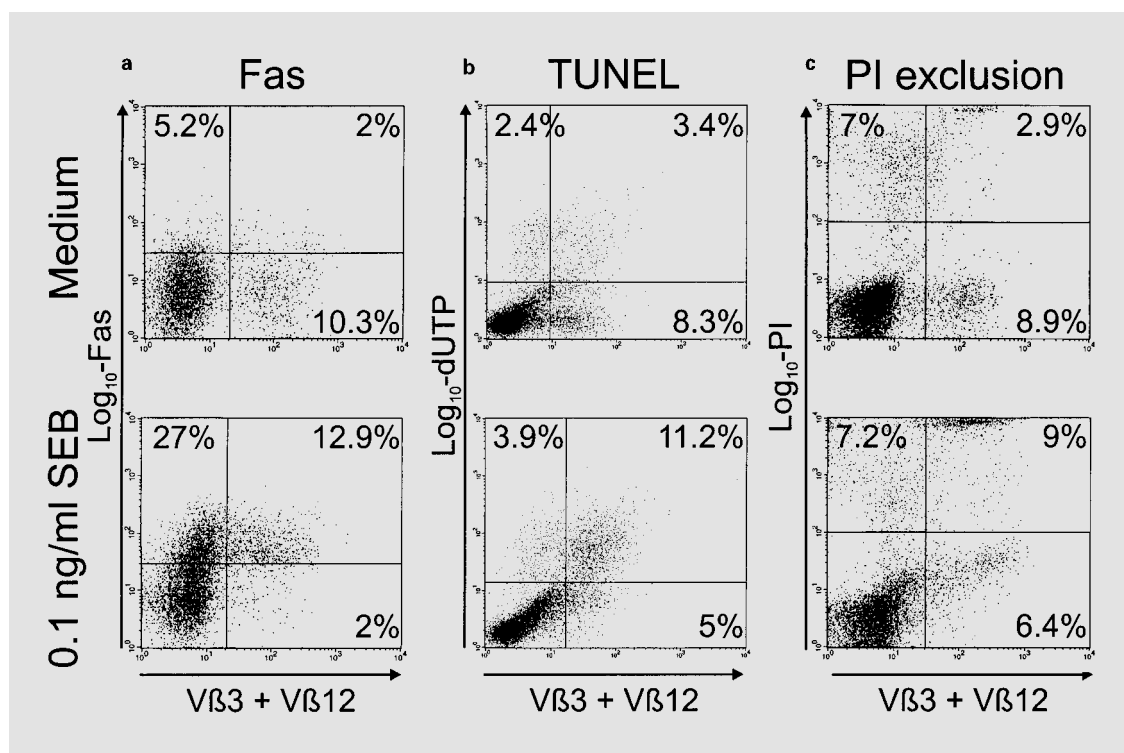


Fig. 5. Analysis of Fas expression and apoptosis on SEB-responsive cells. PBMC were cultured with medium alone or with 0.1 ng/ml SEB. Fas expression (a) on day 2, dUTP incorporation (b) and PI exclusion (c) on day 5 were detected on SEB-responsive V β 3 and V β 12 cells as described in Materials and Methods. Shown are the results of 1 out of 8 similar experiments.

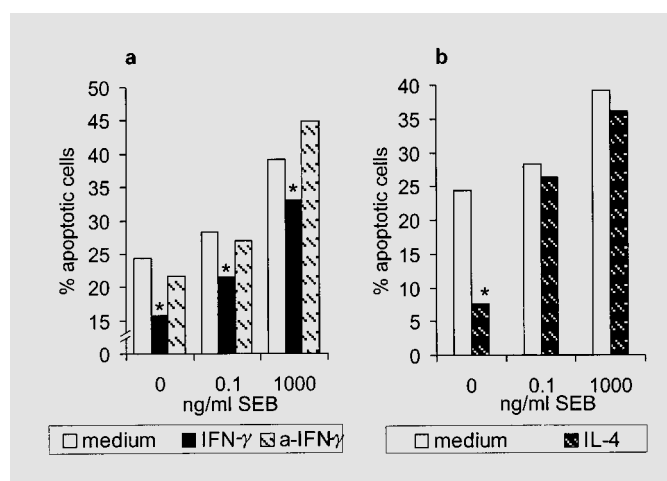


Fig. 6. Effect of IFN- γ and IL-4 on the frequency of apoptotic cells. PBMC were cultured for 10 days with or without 0.1 or 1,000 ng/ml SEB in the absence or presence of 2,000 U/ml IFN- γ , 2 μ g/ml anti-IFN- γ (a) or with 400 U/ml IL-4 (b). Each bar represents the arithmetic mean of 8 experiments of the percentage of apoptotic cells determined by PI staining. Significant differences between medium control and cytokine treated cells: * $p < 0.05$.

Table 2. Influence of the cytokines IFN- γ and IL-4 on Fas expression

Experiment No.	SEB ng/ml	Fas-expressing cells, %		
		medium	IFN- γ	IL-4
1	0	2.4	6.2	10.5
	0.1	21.5	37.2	27.3
	1,000	39.7	57.4	48.9
2	0	1.6	6.6	2.4
	0.1	29.5	49.0	47.2
	1,000	45.3	57.6	65.7

PBMC were cultured for 3 days in the presence of different concentrations of SEB and without or with IL-4 or IFN- γ as indicated. Cells were harvested, washed and stained with anti-Fas antibodies. Two representative experiments are shown.

Discussion

These findings demonstrate a strong dose-dependent association between T cell activation, proliferation, cytokine release and cell death by apoptosis which was preferentially observed in SEB-responsive cells. These *in vitro* effects of SEB on human T cells can be compared to the biphasic *in vivo* effects of SEB on murine T lymphocytes [7–11, 24, 25].

Upregulation of CD25 following SEB stimulation *in vitro* is a well-known phenomenon described in human T cells [31], T cell lines [32] and clones [33] as well as in the murine immune system [34]. However, in a human tonsillar B cell population depleted of T cells SEB selectively upregulates the IL-2R β chain without upregulating the IL-2R α chain [35]. *In vivo* administration of SEB to mice did not always induce CD25 expression prior to T cell death by apoptosis [24, 34]. Therefore, CD25 expression indicates T cell activation, but the IL-2R-mediated pathway does not seem to be essential in superantigen-induced T cell death.

Fas is a 48-kD cell surface antigen of the TNF/nerve growth factor receptor superfamily [36]. Fas expression occurring early after antigen stimulation seems to be a general phenomenon of lymphocyte activation, and Fas engagement can trigger an endogenous endonuclease program which results in cell death by apoptosis [37, 38]. Fas expression and subsequent apoptosis on human T cells upon SEB stimulation occurred in a dose-dependent fashion. Whereas Fas expression was highest on day 2 and declined with time, the rate of apoptotic cells increased with time. The discrepancy between upregulation of Fas and inhibition of apoptosis indicates that additional cellular conditions of Fas expression are necessary for SEB-induced, IFN- γ -modulated cell death. Alternatively, it may be that the Fas ligand was not sufficiently expressed within the initial culture period. We obtained different effects between IFN- γ and IL-4 on SEB-induced apoptosis, but similar effects on the induction of the frequency of Fas-expressing cells. This discrepancy may reflect the fact that additional cellular factors are necessary to modulate apoptotic cell death, or may be due to insufficient Fas expression. One candidate is the Fas ligand, either expressed on cell membranes or in its soluble form. Or Fas is not involved in this process as it has been shown in another cell system [39].

The cytokines IFN- γ and IL-4 play a crucial role in modulating Th1/Th2 responses, with IFN- γ inhibiting the proliferation of Th2 cell activation and IL-4 acting as an autocrine growth factor for the Th2 cell activation [40–

42]. The potential role of cytokines in the modulation of activation-driven cell death is controversial. While IFN- γ was essential for the induction of activation driven cell death of one particular murine Th1 clone [13], it did not play a discernible role in anti-CD3-mediated apoptosis of other murine Th1 and Th2 clones [14]. In human natural killer cells the same cytokines that induce significant IFN- γ production subsequently induce apoptosis of these cells [39]. Therefore, in this cell system endogenous IFN- γ did not seem to protect cells from apoptosis. IL-4 was able to protect murine thymocytes and Th2 T cell clone cells from dexamethasone-induced apoptotic cell death [15, 16]. However, IL-4 augmented cell death in murine T cells stimulated with very high amounts of SEB (10 μ g/ml) [17] and induced apoptosis in some cases of B lineage acute lymphoblastic leukemia [43]. Thus, the modulation of cell death by cytokines depends on a number of factors including the investigated cell system and the stimuli used to induce apoptosis.

We treated human PBMC with the Th1 like cytokine IFN- γ or with the Th2-like cytokine IL-4 prior to SEB stimulation. In IFN- γ -treated PBMC the rate of apoptotic cells decreased upon stimulation with medium alone as well as with all SEB concentrations tested. IL-4 treatment significantly reduced the frequency of apoptotic cells only in the absence or in the presence of low concentrations (≤ 0.001 ng/ml) of SEB; this effect was abolished upon stimulation with higher concentrations (≥ 0.1 ng/ml) of SEB. The fact that IFN- γ reduced the rate of apoptotic cells in PBMC upon SEB stimulation might be important for the regulation of T cell inflammation such as AD. IFN- γ production by intradermal T helper cells is thought to be a major cause for the generation and maintenance of eczema in AD [44, 45]. Clinical improvement is associated with reduced IFN- γ release [22] and increased apoptosis in intradermal T helper cells [23]. Thus, one can put forward the hypothesis that the local production of IFN- γ by intradermal T helper cells inhibits apoptosis of these cells. This might be an additional mechanism through which IFN- γ maintains skin inflammation in this disease.

The effects induced by SEB were dose-dependent with strongest effects seen at 1,000 ng/ml SEB reaching background levels at 0.00001 ng/ml SEB. The greatest progress in the effect of SEB in terms of sequential activation and apoptotic cell was seen at 0.1 ng/ml SEB. Furthermore, this dose was the threshold for the apoptosis-inhibiting effect of IL-4. In PBMC treated with 0.1 and higher doses of SEB IL-4 had no significant effect on the frequency of apoptotic cell death. In mice a dose-dependent effect of

SEB was also observed after administration of superantigens. Whereas low doses of SEB induced a transient state of anergy, higher doses caused long-lasting anergy, and at very high concentrations of SEB 50% of the anergic V β T cells became programmed for apoptosis [11]. Our data suggest that the local environment in terms of superantigen concentrations as well as presence and type of cytokines determine the strength of the T-cell-dependent immune response. It needs to be further investigated, whether the SEB-induced immune reaction in human diseases de-

pends on the amount of superantigen, for example whether the dose of superantigen determines the strength of the inflammatory response in both acute and chronic diseases.

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References

- Marrack P, Kappler J: The staphylococcal enterotoxins and their relatives. *Science* 1990;248:705–711.
- Dellabona P, Peccoud J, Kappler J, Marrack P, Benoist C, Mathis D: Superantigens interact with MHC class II molecules outside of the antigen groove. *Cell* 1990;62:1115–1121.
- Drake CG, Kotzin BL: Superantigens: Biology, immunology, and potential role in disease. *J Clin Immunol* 1992;12:149–162.
- Kappler J, Kotzin B, Herron L, Gelfand E, Bigler R, Boylston A, et al: V β -specific stimulation of human T-cells by staphylococcal toxins. *Science* 1989;244:811–813.
- Choi Y, Kotzin B, Herron L, Callahan J, Marrack P, Kappler J: Interaction of *Staphylococcus aureus* toxin superantigens with human T cells. *Proc Natl Acad Sci USA* 1989;86:8941–8945.
- Marrack P, Blackman M, Kushnir E, Kappler J: The toxicity of staphylococcal enterotoxin B in mice is mediated by T-cells. *J Exp Med* 1990;171:455–464.
- McDonald HR, Baschieri S, Lees RK: Clonal expansion precedes anergy and death of V β 8+ peripheral T-cells responding to staphylococcal enterotoxin B in vivo. *Eur J Immunol* 1991;21:1963–1966.
- Rellahan BL, Jones LA, Kruisbeek AM, Fry AM, Matis LA: In vivo induction of anergy in peripheral V β 8+ T cells by staphylococcal enterotoxin B. *J Exp Med* 1990;172:1091–1100.
- Kawabe Y, Ochi A: Programmed cell death and extrathymic reduction of V β 8+ CD4+ T-cells in mice tolerant to *Staphylococcus aureus* enterotoxin B. *Nature* 1991;349:245–248.
- Webb S, Morris C, Sprent J: Extrathymic tolerance of mature T-cells: Clonal elimination as a consequence of immunity. *Cell* 1990;63:1249–1256.
- Miethke T, Wahl C, Gaus H, Heeg K, Wagner H: Exogenous superantigens acutely trigger distinct levels of peripheral T-cell tolerance/immunosuppression: Dose-response relationship. *Eur J Immunol* 1994;24:1893–1902.
- Renno T, Hahne M, Tschopp J, MacDonald HR: Peripheral T-cells undergoing superantigen-induced apoptosis in vivo express B220 and upregulate Fas and Fas ligand. *J Exp Med* 1996;183:431–437.
- Liu Y, Janeway CA: Interferon γ plays a critical role in induced cell death of effector T-cell: A possible third mechanism of self-tolerance. *J Exp Med* 1990;172:1735–1739.
- Russell JH, Rush BJ, Abrams SI, Wanf R: Sensitivity of T-cells to anti-CD3-stimulated suicide is independent of functional phenotype. *Eur J Immunol* 1992;22:1655–1658.
- Migliorati G, Nicoletti I, Pagliacci C, D'Adamio L, Riccardi C: Interleukin-4 protects double-negative and CD4 single-positive thymocytes from dexamethasone-induced apoptosis. *Blood* 1993;81:1352–1358.
- Zubiaga AM, Munoz E, Huber BT: IL-4 and IL-2 selectively rescue Th cell subsets from glucocorticoid-induced apoptosis. *J Immunol* 1992;149:107–112.
- Ayrolid E, Cannarile L, D'Adamio F, Riccardi C: Superantigen-induced peripheral T-cell deletion: The effects of chemical modification of antigen-presenting cells, interleukin-4 and glucocorticoid hormones. *Immunology* 1995;84:528–535.
- Schlievert PM: The role of superantigens in human diseases. *Curr Opin Infect Dis* 1995;8:170–174.
- Romagnani S: Th1 and Th2 Cells: Role in Human Disorders. Madrid, EAACI, 1995, pp 5–9.
- Hauser C, Wuethrich B, Matter L, Wilhelm J, Sonnabed W, Schopfer K: *Staphylococcus aureus* skin colonization in atopic dermatitis patients. *Dermatologica* 1985;170:35–39.
- Herz U, Bunikowski R, Renz H: Role of T-cells in atopic dermatitis. *Int Arch Allergy Immunol* 1998;115:179–190.
- Grewe M, Bruijnzeel-Koomen C, Schöpf E, Thepen T, Langeveld-Wildschut A, Ruzicka T, et al: A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. *Immunol Today* 1998;19:359–361.
- Morita A, Werfel T, Stege H, Ahrens C, Karmann K, Grewe M, et al: Evidence that singlet oxygen-induced human T helper cell apoptosis is the basic mechanism of ultraviolet-A radiation phototherapy. *J Exp Med* 1997;186:1763–1768.
- Schultz H, Geiselhart A, Sappeler G, Niethammer D, Hoffmann MK: The superantigen *Staphylococcus enterotoxin B* induces a strong and accelerated secondary T-cell response rather than anergy. *Immunology* 1996;87:49–54.
- Gonzalo JA, Biaxeras E, González-García A, George-Chandy A, Van Rooijen N, Martínez AC, et al: Differential in vivo effects of a superantigen and an antibody targeted to the same T-cell receptor. *J Immunol* 1994;152:1597–1608.
- Nicoletti I, Migliorati G, Pagliacci MC, Grignani F, Riccardi C: A rapid and simple method for measuring thymocyte apoptosis by propidium iodide and flow cytometry. *J Immunol Methods* 1991;139:271–279.
- Gorczyca W, Gong J, Darzynkiewicz Z: Detection of DNA strand breaks in individual apoptotic cells by the in situ terminal deoxynucleotidyl transferase and nick translation assays. *Cancer Res* 1993;53:1945–1951.
- Gavrieli Y, Sherman Y, Ben-Sasson SA: Identification of programmed cell death in situ via specific labelling of nuclear DNA fragmentation. *J Cell Biol* 1992;119:493–501.
- Lecoeur H, Gougeon M-L: Comparative analysis of flow cytometric methods for apoptosis quantification in murine thymocytes and human peripheral lymphocytes from controls and HIV-infected persons. Evidence for inference by granulocytes and erythrocytes. *J Immunol Methods* 1996;198:87–99.
- Schmid I, Uittenbogaart CH, Keld B, Giorgi JV: A rapid method for measuring apoptosis and dual-colour immunofluorescence by single laser flow cytometry. *J Immunol Methods* 1994;170:145–157.

- 31 Dannecker G, Mählkecht U, Schultz H, Hoffmann M, Niethammer D: Activation of human T-cells by the superantigen staphylococcus enterotoxin B: Analysis on a cellular level. *Immunobiology* 1994;190:116–126.
- 32 Nielsen M, Svejgaard A, Röpke C, Nordahl M, Odum N: Staphylococcal enterotoxins modulate interleukin 2 receptor expression and ligand-induced tyrosine phosphorylation of the Janus protein-tyrosine kinase 3 (Jak3) and signal transducers and activators of transcription (Stat proteins). *Proc Natl Acad Sci USA* 1995; 92:10995–10999.
- 33 O'Hehir RE, Lamb JR: Induction of specific clonal anergy in human T lymphocytes by *Staphylococcus aureus* enterotoxins. *Proc Natl Acad Sci USA* 1990;87:884–888.
- 34 Gonzalo JA, Moreno de Alboran I, Alés-Martínez JE, Martínez AC, Kroemer G: Expansion and clonal deletion of peripheral T cells induced by bacterial superantigen is independent of the interleukin-2 pathway. *Eur J Immunol* 1992;22:1007–1011.
- 35 Franz A, Bryant A, Farrant J: Staphylococcal enterotoxin B upregulates interleukin-2 receptor β chain expression on tonsillar B cells. *Eur J Immunol* 1993;23:2696–2699.
- 36 Oehm A, Behrmann I, Falk W, Pawlita M, Maier G, Klas C, et al: Purification and molecular cloning of the APO-1 cell surface antigen, a member of the tumor necrosis factor/nerve growth factor receptor superfamily. *J Biol Chem* 1992;267:10709–10715.
- 37 Klas C, Debatin KM, Jonker RR, Krammer PH: Activation interferes with the APO-1 pathway in mature human T-cells. *Int Immunol* 1993;5:625–630.
- 38 Owen-Schaub LB, Yonehara S, Crump WL, Grimm EA: DNA fragmentation and cell death is selectively triggered in activated human lymphocytes by Fas antigen engagement. *Cell Immunol* 1992;140:197–205.
- 39 Ross ME, Caligiuri MA: Cytokine-induced apoptosis of human natural killer cells identifies a novel mechanism to regulate the innate immune response. *Blood* 1997;89:910–918.
- 40 Maggi E, Parronchi P, Manetti R, Simonelli C, Piccini MP, Rugiu FS, et al: Reciprocal regulatory effects of IFN- γ and IL-4 on the in vitro development of human Th1 and Th2 clones. *J Immunol* 1991;148:2142–2147.
- 41 Paul WE, Seder RA: Lymphocyte responses and cytokines. *Cell* 1994;76:241–251.
- 42 Romagnani S: Induction of Th1 and Th2 responses: A key role for the natural immune response? *Immunol Today* 1992;13:379–381.
- 43 Manabe A, Coustan-Smith E, Kumagai M, Behm FG, Raimondi SC, Pui C-H, et al: Interleukin-4 induces programmed cell death (apoptosis) in cases of high-risk acute lymphoblastic leukemia. *Blood* 1994;83:1731–1737.
- 44 Grewe M, Gyufko K, Schöpf E, Krutman J: Lesional expression of IFN- γ in atopic eczema. *Lancet* 1994;343:25–26.
- 45 Thepen T, Langeveld-Wildschut EG, Bihari IC, van Wichen CF, van Reisen FC, Mudde GC, et al: Biphasic response against aeroallergen in atopic dermatitis showing a switch from an initial Th1 response in situ: An immunocytochemical study. *J Allergy Clin Immunol* 1996;97:828–837.

Announcements

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31. Annual Meeting of the German Society for Immunology and of the Nederlandse Vereniging voor Immunologie

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