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Cytokine deviation induced by intrathymic injection of staphylococcal enterotoxin B (SEB)

Abstract

We have previously shown that intrathymic (i.t.) injection of staphylococcal enterotoxin B (SEB) to mice induces both T cell clonal deletion and IL-2-dependent anergy. In the present study, we have used a quantitative RT-PCR to demonstrate that i.t. administration of SEB induced a significant decrease in the levels of the IL-2 and IFN-gamma mRNAs in total splenocytes, from day 7 to day 28 post-injection. I.t. SEB injection also induced a significant increase in the levels both of IL-10 and TGF-beta mRNAs on day 7, leading to a significant

enhance in the IL-10 + TGF-beta/IL-2 + IFN-gamma mRNA ratio on days 7 and 28. By contrast, IL-10 and TGF-beta mRNAs were unchanged after intraperitoneal (i.p.) or subcutaneous (s.c.) SEB injections, although both IL-2 and IFN-gamma mRNA levels were decreased. The cytokine mRNA ratio was enhanced on days 7 and 28 after i.p. injection. Interestingly, a cytokine mRNA ratio of a least 10 in favour of IL-10 plus TGF-beta mRNAs was correlated with the hyporesponsive state observed in vitro after i.t. and i.p. injections. Our results clearly demonstrate that i.t. SEB administration induces a switch from Th1-type to Th2-type cytokine expression in the spleen. The deviation from IL-2 plus IFN-gamma towards IL-10 plus TGF-beta expression could be responsible for the immunoregulatory effect exerted upon SEB-reactive T cells, which is characterized by an IL-2-dependent, specific anergy in vitro. Moreover, it highlights the crucial role of the route of SEB injection in the pattern of cytokine expression.

Full text

INTRODUCTION

Several, non-mutually exclusive mechanisms have been proposed to explain the induction and/or maintenance of specific tolerance to allografts, among which anergy with (or without) an associated down-regulation of TCR and/or accessory molecule expression on the donor-reactive cells, and infectious tolerance are the most relevant in clinical allostransplantation [1-5]. Tolerance induction appears to be highly dependent upon both the route of administration (oral, intravenous, intraperitoneal or subcutaneous) and the amount of antigen. However, intrathymic (i.t.) injection of alloantigens appears to be the most efficient route for inducing modifications of T cell reactivity [6-8]. To date, the precise means by which the route of antigen administration controls the nature and the intensity of the response is not known. Intravenous injection of high doses of antigen into mice results in the apparent deletion or clonal exhaustion of the T cells [9]. An unresponsive state has been achieved in animal model systems by the injection of a superantigen by the i.v. or s.c. routes [10-13]. This unresponsive state appeared to be generated by T cell clonal anergy, in which an initial signal through the T cell receptor, in the absence of costimulation, results in the inability of T cells to produce IL-2, and subsequently to proliferate [14]. However, in other systems there appears to be an initial activation stage before

T cells become unresponsive, as they express activation markers and undergo expansion [15-17].

Staphylococcal enterotoxins belong to a family of bacterial proteins denoted as "superantigen" because of their ability to activate at a high frequency T cells expressing certain beta chains on the TCR variable region (Vbeta). The advantage of the specificity of certain TCR Vbeta domains for endogenous or exogenous superantigens, in order to investigate clonal anergy, has been reported [18-19]. In a previous study, we demonstrated that i.t. injection of staphylococcal enterotoxin B (SEB) induced an initial activation and proliferation of the T cells expressing appropriate Vbeta elements [20]. This early and transient phase of activation was followed by a state of SEB-specific functional T cell inactivation (clonal anergy), both at the central and peripheral compartments. This anergic state was IL-2-dependent, and was more pronounced and more prolonged after i.t. injection than after i.p. or s.c. injections.

Recent experiments have also suggested a possible role for regulatory cytokines in the induction of T cell unresponsiveness [21, 22]. Indeed, during tolerance induction there would seem to be a phase of immune deviation towards the Th2 cytokines, IL-4 and IL-10. This deviation may lead to an IL-10-induced form of anergy or non-responsiveness, and the generation of the recently characterized Th3/T-regulatory T cell subset, which is thought to downregulate antigen-presenting cells [23].

It was reported that injection of the superantigen SEA into Vbeta3/V*11 TCR transgenic mice induced peripheral unresponsiveness in the Vbeta3^{+} T cells and results in IFN-gamma production by suppressor CD4^{-}CD8^{-} splenocytes *in vitro* [24]. SEA stimulation of Vbeta3 transgenic mice was shown to result in high levels of IL-10 in the serum, which were inversely correlated with the amount of IL-2 produced upon restimulation [13]. Again, it has been suggested that IL-10 has a direct effect on T cells by inhibiting IL-2 production [15, 16]. However, a correlative but not a causative role was suggested for IL-10 in the induction/maintenance of T cell proliferative unresponsiveness *in vivo* [13].

In addition to IL-10, TGF-beta has been implicated as a regulatory cytokine produced by the Th3 cells found at sites of chronic antigenic stimulation, especially at mucosal levels [25, 26]. TGF-beta is therefore thought to

contribute to the induction of oral tolerance [27]. TGF-beta is known to block cell cycle progression and may have a direct effect on expression of the IL-2 gene associated with blockage of the IL-2R* chain [28, 29]. Whatever the system used, the molecular mechanisms underlying the induced tolerant state were not clearly delineated and the distinction between anergy and cytokine-mediated suppression is not fully defined.

To evaluate the immune alterations induced in mouse splenocytes following SEB injection by the i.t. route compared to s.c. and i.p. routes, in the present study we have explored the kinetics of the cytokine profile in total splenocytes upon restimulation *in vitro*, in parallel with the kinetics of T cell-specific unresponsiveness. Therefore, we developed a quantitative reverse transcription-polymerase chain reaction (RT-PCR) including internal standard construction to quantify mRNAs of the Th1-type cytokines IL-2 and IFN-gamma, and of the Th2/Th3-type cytokines IL-10 and TGF-beta. This approach provides a precise and sensitive measurement of mRNA levels. Our results indicate that SEB injection induces the decreased expression of IL-2 and IFN-gamma mRNAs, concomitantly with the enhanced expression of IL-10 and TGF-beta mRNAs, but not of IL-4 mRNA. This switch from a Th1-type to a Th2/Th3-type cytokine response parallels the anergic state previously reported.

MATERIALS AND METHODS

Mice and SEB injections

C57BL/6 (H-2^b, Mls^{a-}), 5-6 week-old female mice (IFFA-CREDO, L'Arbresle, France) were used throughout the study.

I.t. injections were carried out under sodium pentobarbital (Sanofi, France) anaesthesia. The thymus lobes were exposed by midline incision over the neck, then injected with 10 mug SEB (Sigma Immunochemical, St Louis, MO, USA) in 10 mul of sterile saline. Intraperitoneal and subcutaneous injections were performed with 50 mug SEB in 0.3 ml and 0.2 ml, respectively. Control mice were injected with the same volumes of sterile saline.

Splenocyte culture for mRNA quantification

The culture medium used throughout the study was RPMI-1640 supplemented with 2 mM-glutamine (Gibco BRL, France), 10 % foetal calf serum (Boehringer Mannheim), 2-beta-mercaptoethanol (5×10^{-5} M), 25 mM Hepes buffer (Gibco BRL, Cergy-Pontoise, France), 100 U/ml penicillin, and 100 mug/ml streptomycin (Gibco BRL).

2.5×10^6 spleen cells were cultured in the presence of 10 mug/ml SEB for 24 hours, then harvested using 1 ml denaturing solution (RNAzol, Bioprobe, Paris) and stored at - 80° C until RNA extraction.

Total RNA extraction

Total RNA was extracted from total splenocytes by homogenisation of 10^6 cells in 1 ml of denaturing solution (RNAazol, Bioprobe, Paris) using the phenol-chloroform extraction technique [30]. Total RNA concentration was determined by measuring the absorbance at 260 nm. To ensure RNA purity, the ratio of absorbance at 260 nm/absorbance at 280 nm should be in the range of 1.8-2.

Oligonucleotides used for cDNA amplification

We designed sets of primers (sense and anti-sense) using Oligo-4 software (Med. Probe, Oslo, Norway) to optimize the annealing temperature and sequence specificity, and to limit self-complementarity (Tables 1 and 2). The oligonucleotide primers were synthesized by Genaxis (France).

Quantitative RT-PCR for IL-2 and IFN-gamma using RNA internal standards

mRNAs for IL-2 and IFN-gamma were quantified using a co-RT-PCR, in which an RNA internal standard was co-reverse transcribed with the RNA samples (I.S.RT-PCR). Subsequently, the resulting sample and internal standard cDNAs were co-amplified by PCR. Internal standards for IL-2 and IFN-gamma were constructed by deletions of 45 and 47 bp sequences, respectively, from the DNA sequences using the mutagenesis assay previously described [31].

Splenocyte RNAs (2 mug) were reverse transcribed in the presence of a known amount of internal standard. The introduction of a known amount of internal standard RNA in the RT assay serves, firstly as an internal control RNA for the RT

reaction, and secondly to generate a standard curve for quantifying the specific target of each mRNA from experimental samples [32].

The optimal amount of internal standard mRNA to introduce in the I.S. RT-PCR had to be determined for each assay. Briefly, various amounts of the internal standard RNA were added to a constant amount of sample RNA (2 mug) (Figure 1A). The experimental and control RNAs and the corresponding cDNAs compete for substrate in reverse transcription and PCR, respectively. For each sample, the amount of the internal standard RNA to be added in the PCR was estimated by direct visualization of the ethidium bromide-stained PCR products from three or four different standard RNA concentrations.

Analysis on gel exhibited two distinct amplification products of the expected size: the first one corresponded to the wild cDNA of the sample, and the second one to the cDNA of the internal standard (Figure 1B). The optimal amount of IL-2 or IFN-gamma mRNA was calculated within the exponential phase by extrapolation from the amount of internal standard added to the reaction mixture (Figure 1C). Results were expressed as the number of specific mRNA molecules per mug of total extrated RNA.

PCR was carried out in the presence of a trace amount of one ^{32}P-labeled 5'primer (2-3 x 10^6 cpm/tube). The mixture was amplified in a PHC3 thermal cycler (Techne, Cambridge, UK) for 36 and 35 cycles, for IL-2 and IFN-gamma, respectively, as previously described [32]. We ensured that both quantification took place in the exponential phase of PCR, and that amplification was similar for both RT products (*i.e.*, known amounts of internal control and cDNA samples) by plotting the amount of radioactivity (cpm) against the number of PCR cycles on sequential samples from 22 to 36 cycles.

Results are expressed as the number of specific mRNA molecules per mug of total extracted RNA.

Quantitative LightCycler™ SYBR™ Green I RT-PCR for IL-10 and TGF-beta

We have recently adapted the LightCycler™ technology (Roche, Boeringer Mannheim) in order to quantify IL-10 and TGF-beta mRNAs, as described [33]. The results obtained for IL-2 and TGF-beta mRNA quantification by I.S. and LightCycler™ techniques were similar.

Both at the end of each amplification cycle and all along each melting curve analysis, fluorimetric measures were registered. A single external standard curve (log concentration *versus* number of cycles) was obtained from two-fold dilutions (from 1/2 to 1/32) of a specific standard DNA amplification product. Results were expressed as arbitrary units (a.u.).

The melting curve analysis allows the detection of undesired, non-specific products or dimers in the amplification products. For complementary verification of the length of the PCR product, they were separated by electrophoresis onto a 1.5 % agarose gel.

Cytokine quantification by ELISA

IL-2 and IL-10 levels in splenocyte supernatants were flasted by enzyme-linked immunosorbent assays, using commercially available kits (Quantitine M mouse IL-2 and Quantitine M mouse IL-10, R&D Systems). The calibration range was 0 to 1,000 pg/ml. The test were accurate down to 3.0 pg/ml for IL-2 and 4.0 pg/ml for IL-10.

Statistical analysis

Results were expressed as indicated in each experiment and recorded as the mean value ± SEM. The non-parametric test was used throughout the study. Differences with p values lower than 0.05 were considered significant.

RESULTS

In the present study, we have investigated the pattern of cytokine mRNA expression in total splenocytes after restimulation for 24 hours with 10 mug/ml of SEB *in vitro*. Groups of 6 mice were injected with SEB by the i.t. (10 mug), i.p. (50 mug) or s.c. (50 mug) routes, since these doses of SEB were previously shown to be the most efficient for inducing immunological alterations according to the route of administration [20].

The specific mRNA levels were quantified on total splenocytes using the RT-PCR with internal standard (I.S. RT-PCR) for the IL-2 and IFN-gamma, and the LightCycler™ technique for IL-10 and TGF-beta. None of the cytokine mRNAs

investigated in the present study were detectable in the thymocyte extracts by either RT-PCR techniques.

mRNA levels in total splenocytes

In all cases, the 28S RNA levels in total splenocyte from control and SEB-treated mice were similar, confirming that similar amounts of RNA were introduced (Figure 2). Again, the beta-actin mRNA levels were not significantly different in extracts from control and SEB-treated mice, whatever the route of injection (in the range of 20.5 to 45.7 a.u.), indicating that SEB-injection did not alter the overall expression of mRNAs in splenocytes.

Quantitative determination of IL-2 and IFN-gamma mRNAs

IL-2 and IFN-gamma mRNAs were quantified on total splenocyte samples from 6 SEB-treated and 6 saline-injected control mice on days 2, 7, and 28 after SEB injection.

The mean amounts of IL-2 mRNA in control mice were not significantly different on the three days of experiments (2, 7, and 28) after i.t., i.p. and s.c. treatments, and ranged from 12×10^5 to 18×10^5 molecules/mug of RNA (Figure 3). The mean amounts of IFN-gamma mRNA in control mice were not significantly different on days 2, 7, and 28 after i.t. and i.p. treatments, and ranged from 79.1×10^5 to 193.2×10^5 molecules/mug of RNA. Unexpectedly, the mean amount of IFN-gamma mRNA progressively increased from day 2 until day 28 following s.c. injection of saline (control mice), from $45.6 \pm 12.8 \times 10^5$ to $193.3 \pm 37.8 \times 10^5$ molecules/mug of RNA ($p < 0.05$).

Figure 3 shows that following i.t. injection, the number of IL-2 mRNA molecules decreased 8-fold at days 7 and 28 in SEB-treated mice ($3.5 \pm 1.1 \times 10^5$ *versus* $27.7 \pm 3.3 \times 10^5$ molecules/mug of RNA in control mice, $p < 0.001$ on day 7; $3.1 \pm 1.2 \times 10^5$ *versus* $13.9 \pm 2.1 \times 10^5$ molecules/mug of RNA in control mice, $p < 0.001$ on day 28). In parallel, the number of IFN-gamma mRNA molecules was dramatically decreased from day 2 ($11.0 \pm 2.1 \times 10^5$ *versus* $193.2 \pm 17.4 \times 10^5$ molecules/mug of RNA in control mice, $p < 0.01$) until day 28 ($3.8 \pm 1.5 \times 10^5$ molecules/mug of RNA *versus* $142 \pm 37.2 \times 10^5$ in control mice, $p < 0.01$).

Following i.p. injection of SEB, the number of IL-2 mRNA molecules was significantly decreased from day 2 (2.7-fold, $p < 0.01$ *versus* control mice) until day 28 (4.5-fold, $p < 0.001$) (Figure 3). In parallel, the amounts of IFN-gamma mRNA in splenocytes from SEB-treated mice were significantly decreased from day 2 (8.9-fold, $p < 0.001$) until day 28 (17.7-fold, $p < 0.01$).

Figure 3 also shows that following s.c. injection of SEB, the amount of IL-2 mRNA was decreased by 3.4-nimes only at day 7) $3.7 \pm 1.1 \times 10^5$ molecules/mug of RNA in seb-treated mice *versus* $12.4 \pm 2 \times 10^5$ in control mice, $p < 0.01$), then returned to control level on day 28. By contrast, the amount of IFN-gamma mRNA was significantly decreased at days 7 (4.6-fold, $p < 0.01$) and 28 (13.3-fold, $p < 0.001$).

In conclusion, SEB injection resulted in a significant, early decrease in the amount of IL-2 mRNA at day 2 only in the case of the i.p. route, as summarized in Figure 5. At day 7, this decrease was dramatic for the three routes of injection, although significantly more pronounced after i.t. and i.p. than s.c. injections ($p < 0.05$ on days 7 and 28). Lastly, on day 28, the level of IL-2 mRNA had returned to the level observed on day 2 only after s.c. injection of SEB. In parallel, a significant decrease in IFN-gamma mRNA occured as soon as day 2 following i.t. or i.p. injections of SEB, then remained similar for the three routes of injection until day 28.

Quantitative determination of TGF-beta and IL-10 mRNAs

TGF-beta and IL-10 mRNAs were quantified in total splenocytes from SEB-injected mice on days 2, 7, and 28 post-SEB injection.

The levels of IL-10 mRNA in control mice were similar on the three days of experiment after i.t. injection of saline (Figure 4). Unexpectedly, the levels reached in control mice were significantly higher at days 7 and 28 following i.p. or s.c. injection than at day 2 ($p < 0.05$). By contrast, the levels of TGF-beta mRNA were similar in all control groups and ranged from 20.2 to 38.5 arbitrary units.

After i.t. injection of SEB, the levels both of IL-10 and of TGF-beta mRNAs were significantly increased on days 2 (15.7 a.u. *versus* 3.6 a.u. in control mice, $p < 0.001$, for IL-10; 56.6 a.u. *versus* 25.6 a.u. in control mice, $p < 0.01$, for TGF-beta), and 7 (21.7 a.u *versus* 12.3 a.u. in control mice, $p < 0.001$ for IL-10; 22.3 a.u. *versus*

64.8 a.u. in control mice, $p < 0.001$, for TGF-beta), then returned to the corresponding control levels on day 28 (Figure 4).

After i.p. injection of SEB, the level of IL-10 mRNA was significantly decreased on day 2 (5.1 a.u. *versus* 14.5 a.u. in control mice, ($p < 0.001$), whereas the level of TGF-beta mRNA significantly increased (94.3 a.u. in SEB-treated mice *versus* 34.9 a.u. in control mice ($p < 0.01$).

After s.c. injection, the levels of IL-10 mRNA in SEB-treated mice were similar to the corresponding control group throughout the experiment (Figure 4). Unexpectedly, the level of TGF-beta mRNA was significantly decreased on day 7 in SEB-treated mice (15.1 a.u. *versus* 32.2 a.u. in control mice, $p < 0.01$).

To summarize, only the i.t. route for SEB injection resulted in any significant increase in the amounts both of IL-10 and TGF-beta mRNAs, as early as day 2 and until day 7. This increase was significantly more pronounced after i.t. that i.p. or s.c. injections at day 2, and was still significantly higher at day 7 (Figure 5). I.p. injection of SEB resulted in an early but transient (day 2) increase in the level of TGF-beta mRNA, but a decrease in the level of IL-10 mRNA. By contrast, s.c. injection of SEB induced a significant decrease in TGF-beta mRNA levels at day 7.

IL-2 and IL-10 protein expression

IL-2 and IL-10 cytokines released by total splenocytes from SEB-treated and control mice (3 mice per group) were assayed by ELISA on the culture supernatants after restimulation for 24 hours with 10 mug/ml of SEB *in vitro*.

IL-2 release in supernatants was signicantly decreased from day 2 to day 28, whatever the route of SEB injection ($p < 0.01$ *versus* the corresponding control group), although to a greater extent at day 28 (Figure 6). Concomitantly, the IL-10 concentrations were significantly increased ($p < 0.01$ *versus* control group). However, the IL-10 release was significantly more increased after i.t. than i.p. injection ($p < 0.01$ at days 2, 7, and 28) or s.c. injection ($p < 0.05$ at day 2, and $p < 0.01$ both at days 7 and 28).

Cytokine pattern and anergy induced in splenocytes following SEB-injection of mice

To explore the possible parallelism between the pattern of cytokine mRNA expression in total splenocytes, and the SEB-specific unresponsive state that we previously observed *in vitro* following SEB injection of mice [20], we calculated the ratio of mRNA levels of the Th2/Th3-type cytokines IL-10 and TGF-beta to the mRNA levels of the Th1-type cytokines IL-2 and IFN-gamma.

Figure 7 (top right) shows that 7 days following i.t. or i.p., but not s.c. injection of SEB, the proliferative response of splenocytes to 10 mug of SEB *in vitro* was significantly decreased ($p < 0.001$ *versus* pre-treatment group).

At day 28 after SEB injection (Figure 7 bottom right), the unresponsiveness induced by i.t. injection of SEB was still significant ($p < 0.01$), whereas a rebounding, significant hyper-responsiveness was observed after i.p. injection ($p < 0.01$). Lastly, the proliferative response was not altered after s.c. injection of SEB.

Figure 7 (left) clearly shows that the cytokine mRNA ratio at day 7 was significantly enhanced both after i.t. (mean ratio = 12.6) and after i.p. (mean ratio = 10.2) injections of SEB, and significantly higher that after s.c. injection (mean ratio = 2.4, $p < 0.01$ for i.t. and i.p. routes *versus* s.c. route). The former elevated ratios were both correlated with functional unresponsiveness.

It is very interesting to note that on day 28, the mean mRNA ratio calculated from i.t. injected mice remained at a similar level (mean ratio = 9.2), and was correlated with a lasting state of anergy. By contrast, after i.p. injection, the mean ratio had returned to a low level (mean ratio = 3.5), and was correlated with the loss of the unresponsive state, although both IL-2 and IFN-gamma mRNA levels were still dramatically decreased, as earlier reported in Figure 3. The steady, low mRNA cytokine ratio calculated after s.c. injection of SEB paralleled the absence of hypo- or hyper-response to SEB observed in the proliferative assay *in vitro*.

From these results, we can assume that only an IL-10 + TGF-beta mRNA/IL-2 + IFN-gamma mRNA ratio higher than 4, and may be around 10, is correlated with SEB-specific unresponsiveness.

DISCUSSION

We have previously shown that SEB injection into mice induced an SEB-specific, IL-2-dependent unresponsive state in total splenocytes restimulated with SEB *in vitro* [20]. The aim of the present study was to investigate the pattern of cytokine expression induced by SEB injection. Indeed, the balance between the Th1-type cytokines IL-2 and IFN-gamma, and the Th2/Th3 cytokines TGF-beta and IL-10 may play a major role in the induction of T cell unresponsiveness. We focused on mRNA levels in total splenocytes, since we could not detect mRNA for these cytokines in thymocytes using our techniques.

The properties of tolerized cells have not yet been well characterised due to the difficulty of recovering adequate numbers of these cells. In one case, Vbeta TCR transgenic mice given an injection of Mls-incompatible spleen cells showed that clonal anergy played a role in the tolerance process [34]. By contrast, other investigators using a similar Mls incompatibility in a non-transgenic model concluded that anergy induction does not play a role in this system [11]. To study the cytokine balance after i.t. injection of antigen, which appears to be the most efficient route for inducing alterations of T cell reactivity [6-8], we injected a superantigen as a model of amplified T cell activation in mice. Since *in vitro* models have clearly established an association between repressed IL-2 mRNA expression and a non-responsive state [35], we explored the mRNA levels of cytokines induced in total splenocytes obtained on days 2, 7, and 28 following SEB injection after *in vitro* restimulation for 24 hours with SEB. This sensitive method for investigating cytokine expression avoids prolonged *in vitro* culture, which can introduce artefacts, as well as general, new cytokine-producing cells. It is also a suitable compromise for quantification both of mRNA and of proteins for the four cytokines tested.

Our results confirm that SEB injection induces a significant decrease in IL-2 mRNA on day 7, whatever the route of injection, which is in agreement with other reports [36]. This decrease was still evident 4 weeks after i.t. and i.p. injections, and was significantly more substantial after i.t. and i.p. than s.c. injections, although the dose of SEB injected by the i.t. route was 5 times lower than for the i.p. or s.c. routes. In parallel, we also showed a dramatic reduction in IFN-gamma mRNA levels in contrast with normal IFN-gamma expression reported by the same authors. This reduction was early, and durable whatever the route of SEB injection, although it was delayed after s.c. injection.

Several studies have indicated that regulatory cytokines may contribute to the overall tolerant state *in vivo* [21]. A role for TGF-beta in the maintenance of the tolerant state has been shown in a number of animal models. Mice rendered tolerant by oral ingestion of antigen develop $CD8^+$ regulatory T cells that express varying amounts of TGF-beta, IL-10, and IL-4 [37]. A role for TGF-beta in the negative regulation of the immune response, possibly by actively suppressing or immune deviating reactive T cells has been reported [27]. In recent studies, $CD4^+$ [15] and $CD8^+$ [16] T cells rendered tolerant *in vivo* by chronic antigenic stimulation were shown to express the cytokine IL-10 after restimulation *in vitro*. The involvement of IL-10 tolerance has also been demonstrated in a superantigen model involving multiple injections of SEA into a Vbeta transgenic mouse [13], with an inverse correlation between the amounts of IL-2 and IL-10 elicited.

Our results confirm that SEB-induced anergy is not IL-2-dependent, nor related to IL-10 release, since the decrease in IL-2 expression and/or the increase in IL-10 expression did not parallel anergy. It can be postulated that the decrease in IL-2 expression is a pre-requisite for, but is not sufficient to lead to an anergic state, and that IL-10 plays a role in the prolongation of the anergic state. It seems likely that other cytokines play a role in the induction and in the maintenance of anergy, especially TGF-beta. In our model, only after i.t. injection a significant, early increase in TGF-beta mRNA level was observed and still evident on day 7, and paralleled the increase in IL-10 mRNA levels. Taken together, these results clearly show that the effect of SEB injection upon Th1-like cytokines (decrease) does persist at the time when the effect of Th2-like cytokines expression (increase) has disappeared.

These data pose an intriguing question as to the nature of the relationship between the suppression and the anergy: were they in fact separate components of the tolerance? It has been suggested that the immune response (T cell anergy *versus* activation) following alloantigen administration is closely related to the balance between the Th2 and Th1 cytokines rather than the increase in the former or the decrease in the latter cytokines. In many cases it has been reported than the induction of peripheral tolerance to alloantigen is accompanied by a decrease in the production of cytokines such as IL-2 and IFN-gamma, yet an increased production of cytokines such as IL-10 and IL-4 [38]. Although the Th1/Th2 paradigm has proven a potent conceptual framework for the operational classification of T cells, studies have raised doubts about its

validity in the induction of allograft acceptance and peripheral transplantation tolerance. To further investigate the role of cytokine balance in anergy, it has been postulated that the Th2/Th1 cytokine ratio may be useful for looking at skewing of T cell cytokine profiles. Taking into account the critical cytokines reported in the induction of allograft acceptance, we have studied the ratio of IL-4 mRNA plus TGF-beta mRNA to IL-2 plus IFN-gamma mRNA, as the balance between Th2/Th3-type and Th1-type cytokines. In our model, we have demonstrated a close relationship between hypo-responsiveness *in vitro* (functional anergy) and an increase in the Th2/Th1 ratio. The inhibition of the proliferative response on day 28 was only observed after i.t. SEB injection, which correlates with a mean Th2/Th1 ratio of at least 10. The profoundness of the unresponsiveness seen at day 7 after i.t. injection is clearly related to the combination of an IL-2-dependent anergy and a suppression mediated by IL-10 and TGF-beta. This is in agreement with Groux *et al*. who have shown that stimulation of human naive T cells in the presence of IL-10 can facilitate the induction of an anergic-like state [39]. Although IL-2 and IL-10 appeared to be critical, the expression of IL-10, and probably of TGF-beta, may prove to be more valuable than the failure of IL-2 expression, to achieve tolerance.

In our model, to clearly identify the relationship between the increase of both IL-10 and of TGF-beta production, and the anergic state of SEB-reactive T cells, the abrogation of the induction of specific unresponsiveness to SEB should be investigated by *in vivo* injection of neutralizing antibody specific to IL-10 or TGF-beta.

Whereas IL-10 mRNA levels were correlated with the cytokine release as determined by ELISA, a decrease in IL-2 release was concomitant with an unchanged IL-2 mRNA expression on day 2. We can postulate that this discrepancy is related to IL-2 consumption.

Comparison of the different routes of administration of SEB clearly shows that the i.t. route was the most efficient for inducing alterations of T cell reactivity, which may be accounted for by differences in SEB distribution as previously demonstrated. Indeed, we have previously shown that SEB migrates from the central to the peripheral compartment after i.t. injection [20], suggesting that i.t. SEB injection may lead to a combination of central and peripheral alterations of the cytokine balance, although we were not able to quantify the cytokine mRNAs in the thymocyte extracts. It has been suggested that recent thymic

emigrants, but not mature T cells, were able to develop into functional T regulatory cells in the presence of peripheral alloantigen [40]. Therefore, we can not exclude an effect of recently emigrant thymic regulatory T cells on peripheral cytokine expression. Indeed, evidence has recently emerged that during tolerance induction there is a phase of immune deviation towards Th2 cytokines and generation of a Th3/ T-regulatory T cell subset [41].

CONCLUSION

Our results clearly show that intrathymic injection of SEB induces an increase in the mRNA expression of the Th2-type cytokines IL-10 and TGF-beta, which parallels a decrease in the expression of the Th1-type cytokines IL-2 and IFN-gamma. Our data suggest that a T regulatory T cell subset was generated upon SEB stimulation *in vivo*. Moreover, the intrathymic route was the most efficient for inducing a long-lasting immunodeviation towards the Th2-type cytokines. We postulate that an elevated Th2/Th1 cytokine ratio combined with a decrease in IL-2 mRNA is critical in the maintenance of the anergic state. Therefore, the modulation of the Th2/Th1 cytokine balance may be a way of regulating the tolerance/activation balance.

References

1. Wells A D, Li X C, Li Y, Walsh M C, Zheng X X, Wu Z, Nunez G, Tang A, Sayegh M, Hancock W W, Strom T B, Turka L A. 1999. Requirement for T cell apoptosis in the induction of peripheral transplantation tolerance. *Nat Med.* 5: 1303.

2. Gillanders W E, Arima T, Tu F, Hansen T H, Flye M W. 1997. Evidence for clonal deletion and clonal anergy after intrathymic antigen injection in a transplantation model. *Transplantation* 64: 1159.

3. Braun M Y, McCormack A, Webb G, Batchelor J R. 1993. Evidence for clonal anergy as a mechanism responsible for the maintenance of transplantation tolerance. *Eur. J. Immunol.* 23: 1462.

4. Reemtsen B L, Kato H, Wang T S, Busuttil R W, Kupiec-Weglinski J W, Goss J A. 1999. Intrathymic immunomodulation and the "infectious" tolerance pathway in allograft recipients. *J. Surg. Res.* 84: 1.

5. Onodera K, Lehmann M, Volk H D, Sayegh M H, Kupiec-Weglinski J W. 1997. CD4-targeted therapy induces "infectious" tolerance to cardiac allografts in sensitized rat recipients. *Transplant. Proc.* 29: 1907.

6. Goss J A, Nakafusa Y, Roland C R, Hickey W F, Flye M W. 1994. Immunological tolerance to a defined myelin basic protein antigen administrated intrathymically. *J. Immunol.* 153: 3890.

7. Goss J A, Nakafusa Y, Yu S, Flye M W. 1993. Intrathymic injection of donor alloantigens induces specific tolerance to cardiac allografts. *Transplantation.* 56: 166.

8. Chen W, Sayegh M H, Khoury S J. 1998. Mechanisms of acquired thymic tolerance *in vivo*: intrathymic injection of antigen induces apoptosis of thymocytes and peripheral T cell anergy. *J. Immunol.* 160: 1504.

9. Moskophidis D, Laine E, Zinkernagel R M. 1993. Peripheral clonal deletion of antiviral memory $CD8^+$ T cells. *Eur. J. Immunol.* 23: 3306.

10. Rammensee H G, Kroschewski R, Frangoulis B. 1989. Clonal anergy induced in mature V beta 6^+ T-lymphocytes on immunizing Mls-1b mice with Mls-la expressing cells. *Nature.* 339: 541.

11. Webb S R, Hutchinson J, Hayden K, Sprent J. 1994. Expansion/deletion of mature T cells exposed to endogenous superantigens *in vivo. J. Immunol.* 152: 586.

12. McCormack J E, Callahan J E, Kappler J, Marrack P C. 1993. Profound deletion of mature T cells *in vivo* by chronic exposure to exogenous superantigen. *J. Immunol.* 150. 3785.

13. Sundstedt A, Hoiden I, Rosendahl A, Kalland T, van Rooijen N, Dohlsten M. 1997. Immunoregulatory role of IL-10 during superantigen-induced hyporesponsiveness *in vivo*. *J. Immunol.* 158: 180.

14. Schwartz R H, Mueller D L, Jenkins M K, Quill H. 1989. T cell clonal anergy (Review). *Cold Spring Harb Symp Quant Biol.* 54: 605.

15. Buer J, Lanoue A, Franzke A, Garcia C, von Boehmer H, Sarukhan A. 1998. Interleukin 10 secretion and impaired effector function of major histocompatibility complex class II-restristed T cells anergized *in vivo. J. Exp. Med.* 187: 177.

16. Tanchot C, Guillaume S, Delon J, Bourgeois C, Franzke A, Sarukhan A, Trautmann A, Rocha B. 1998. Modifications of $CD8^+$ T cell function during *in vivo* memory or tolerance induction. *Immunity*. 8: 581.

17. Adler B, Weber G F, Cantor H. 1998. Activation of T cells by superantigen: cytokine production but not apoptosis depends on MEK-1 activity. *Eur. J. Immunol.* 28: 3749.

18. Dowd J E, Jenkins R N, Karp D R. 1995. Inhibition of antigen-specific T cell activation by staphylococcal enterotoxins. *J. Immunol*. 154: 1024.

19. Seth A, Stern L J, Ottenhoff T H, Engel I, Owen M J, Lamb J R, Klausner R D, Wiley D C. 1994. Binary and ternary complexes between T cell receptor, class II MHC and superantigen *in vitro. Nature*. 369: 324.

20. Goettelfinger P, Roussin R, Lecerf F, Berrih-Aknin S, Fattal-German M. 2000. T cell deletion and unresponsiveness induced by intrathymic injection of staphylococcal enterotoxin B. *Transpl. Immunol.* 8: 39.

21. Laliotou B, Duncan L, Dick A. D. 1999. Intranasal administration of retinal antigens induces transient T cell activation and apoptosis within drainage lymph nodes but not spleen. *J. Autoimmun.* 12: 145.

22. Onodera K, Volk H D, Ritter T, Kupiec-Weglinski J W. 1998. Thymus requirement and antigen dependency in the "infectious" tolerance pathway in transplant recipients. *J. Immunol*. 160: 5765.

23. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries J E, Roncaroco M G. 1997. A $CD4^+$ T cell subset inhibits antigen-specific T cell responses and prevents colitis. *Nature.* 389: 737.

24. Cauley L S, Cauley K A, Shub F, Huston G, Swain S L. 1997. Transferable anergy: superantigen treatment induces $CD4^+$ T cell tolerance that is reversible and

requires $CD4^{-}CD8^{-}$ cells and interferon gamma. *J. Exp. Med.* 186: 71.

25. Powrie F, Carlino J, Leach M W, Mauze S, Coffman R L. 1996. A critical role for transforming growth factor-beta but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB (low) $CD4^{+}$ T cells. *J. Exp. Med.* 183: 2669.

26. Strober W, Kelsall B, Fuss I, Marth T, Ludviksson B, Neurath M. 1997. Reciprocal IFN-gamma and TGF-beta response regulate the occurence of mucosal inflammation (Review). *Immunol Today.* 18: 61.

27. Miller A, Lider O, Roberts A B, Sporn M B, Weiner H L. 1992. Suppressor T cells generated by oral tolerization to myelin basic protein suppress both *in vitro* and *in vivo* immune response by the release of transforming growth factor beta after antigen-specific triggering. *Proc. Natl. Acad. Sci. U S A.* 89: 421.

28. Hocevar B A, Howe P H. 1998. Mechanisms of TGF-beta-induced cell cycle arrest (Review). *Miner Electrolyte Metab*. 24: 131.

29. Brabletz T, Pfeuffer I, Schorr E, Siebelt F, Wirth T, Serfling E. 1993. Transforming growth factor beta and cyclosporin A inhibit the inductible activity of the interleukin-2 gene T cells through a noncanonical octamer-binding site. *Mol. Cell. Biol*. 13: 1155.

30. Guyon T, Levasseur P, Truffault F, Cottin C, Gaud C, Berrih-Aknin S. 1994. Regulation of acetylcholine receptor alpha subunit variants in human myasthenia gravis. Quantification of steady-state levels of messenger RNA in muscle biopsy using the polymerase chain reaction. *J. Clin. Invest*. 94: 16.

31. Guyon T. 2000. Préparation du contrôle interne sous forme d'ARN. In: Berrih-Aknin S. eds. *PCR Quantitative*. Paris: Les Editions Inserm, Technique en immunologie, 19.

32. Goettelfinger P, Poëa S, Wakkach A. 2000. Quantification des produits d'amplification par une technique radioactive. In: Berrih-Aknin S. eds. *PCR Quantitative.* Paris: Les Editions Inserm, Technique en immunologie, 49.

33. Jacquey F. 2000. PCR et RT-PCR quantitatives en cinétique et détection de mutations. In: Berrih-Aknin S. eds. *PCR Quantitative*. Paris: Les Editions Inserm, Technique en immunologie, 79.

34. Bhandoola A, Cho E A, Yui K, Saragovi H U, Greene M I, Quill H. 1993. Reduced CD3-mediated protein tyrosine phosphorylation in anergic $CD4^+$ and $CD8^+$ T cells. *J. Immunol*. 151: 2355.

35. Telander D G, Malvey E N, Mueller D L. 1999. Evidence for repression of IL-2 gene activation in anergic T cells. *J. Immunol.* 162: 1460.

36. Koide Y, Uchijima M, Yoshida A, Yoshida T O. 1996. Effect of staphylococcal enterotoxin B-induced anergy on cytokine gene expression: anergy-sensitive and resistant mRNA expression. *J. Interferon Cytokine Res.* 16: 225.

37. Wilbanks G A, Streilein J W. 1992. Fluids from immune privileged sites endow macrophages with the capacity to induce antigen-specific immune deviation *via* a mechanism involving transforming growth factor-beta. *Eur. J. Immunol*. 22: 1031.

38. Dallman M J, Wood K J, Hamano K, Bushell A R, Morris P J, Wood M J, Charlton H M. 1993. Cytokines and pe-ripheral tolerance to alloangiten (Review). *Immunol. Rev.* 133: 5.

39. Groux H, Bigler M, de Vries J E, Roncarolo M G. 1996. Interleukin-10 induces a long-term antigen-specific anergic state in human $CD4^+$ T cells. *J. Exp. Med*. 184: 19.

40. Modigliani Y, Coutinho A, Pereira P, Le Douarin N, Thomas-Vaslin V, Burlen-Defranoux O, Salaun Y, Bandeira A. 1996. Establishment of tissue-specific tolerance is driven by regulatory T cells selected by thymic epithelium. *Eur. J. Immunol.* 26: 1807.

41. Cobbold S, Waldmann H. 1998. Infectious tolerance (Review). *Curr. Opin. Immunol*. 10: 518.

Figures

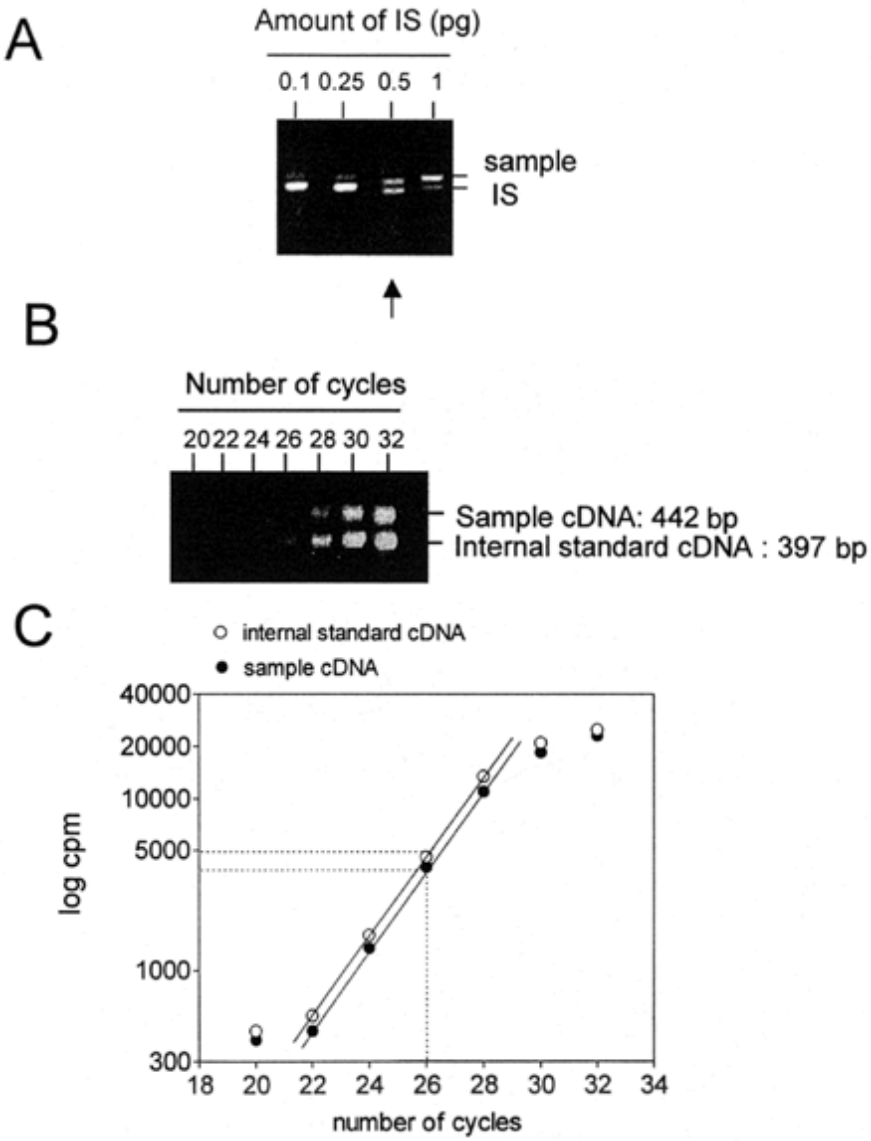


Figure 1
Illustration of RT-PCR using an internal standard for IL-2 mRNA quantification.

(A) Determination of a suitable amount of internal standard to add in the RT-PCR from the equal intensity of internal standard and sample cDNA bands (0.5 pg, as indicated).

(B) RT-PCR.

At various times, aliquots were taken off and loaded on to 2.5% agarose electrophoresis gel.

(C) Amplification curve obtained by plotting the number of cycles against cpm (logarithm scale).

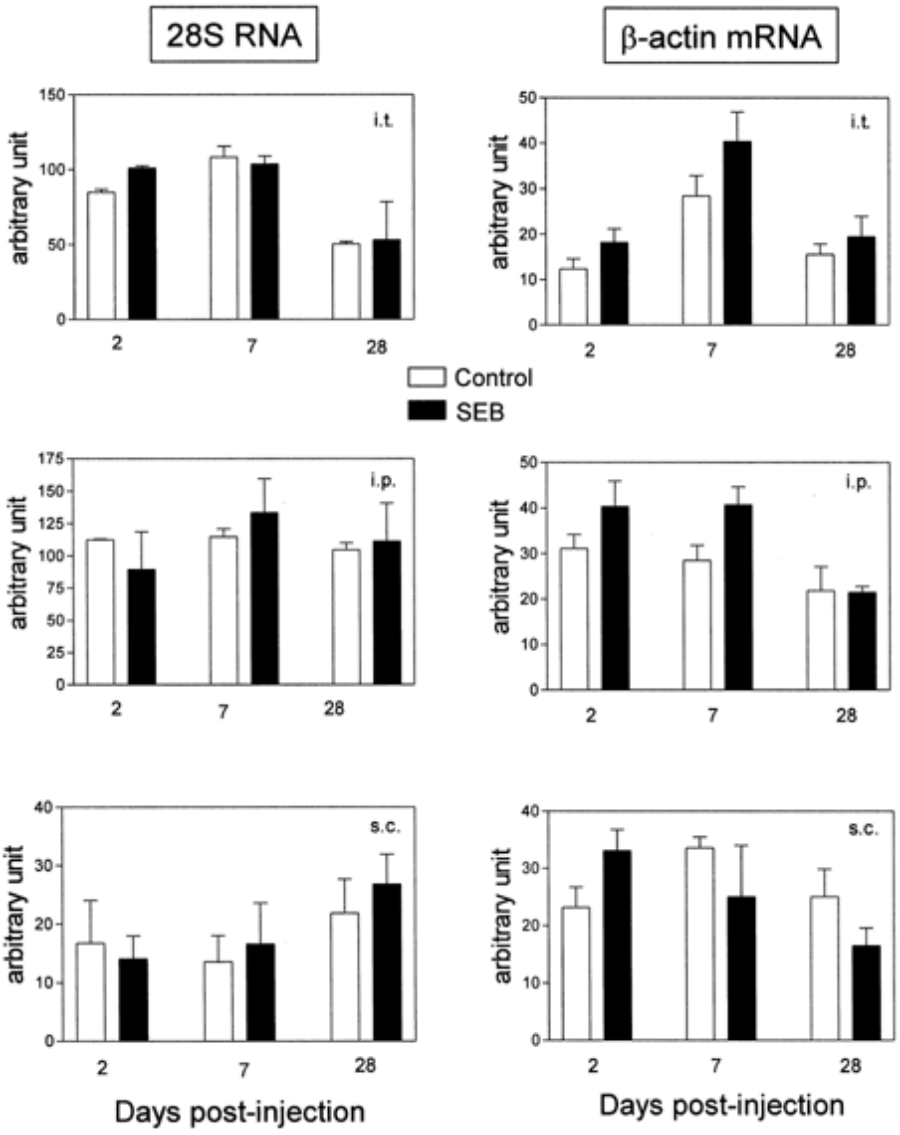


Figure 2
28S RNA (left panels) and beta-actin mRNA (right panels) expression in total splenocytes from mice injected with SEB by the intrathymic (i.t.), intraperitoneal (i.p.), and subcutaneous (s.c.) routes,

on days 2, 7, and 28 after injection, using the LightCycler™ technique.

Splenocytes were restimulated for 24 hours with 10 µg/ml of SEB *in vitro*. Results are expressed as arbitrary units and reported as the mean value ± SEM. n = 6 mice/group.

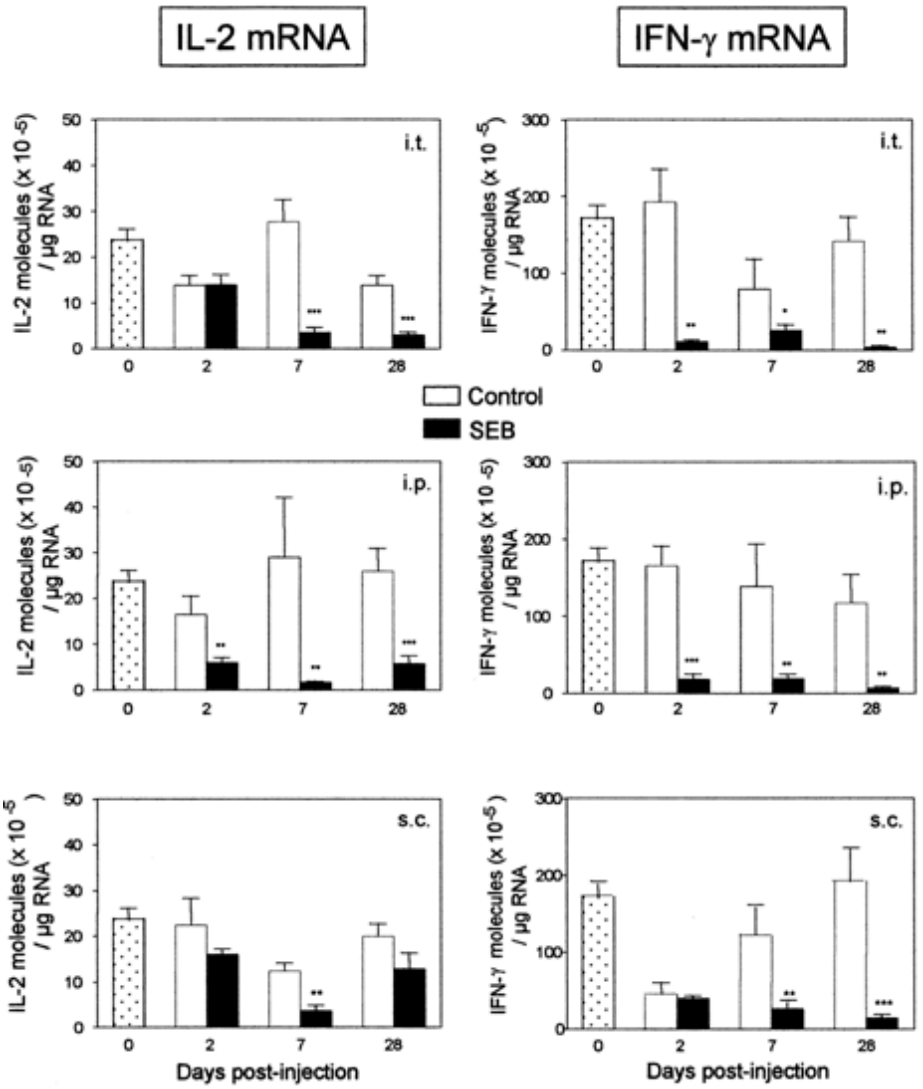


Figure 3
IL-2 (left-panels) and IFN-gamma (right panels) mRNA expression in total splenocytes from saline (control) or SEB-injected mice on days 2, 7, and 28 by the intrayhymic (i.t.), intraperitoneal (i.p.), and subcutaneous (s.c.) routes: splenocytes were

restimulated for 24 hours with 10 mug/ml of SEB *in vitro*.

Results are expressed as the number of specific mRNA molecules x 10^{-5} per µg of total cell RNA, ± SEM.

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ *versus* controls. N = 6 mice/group.

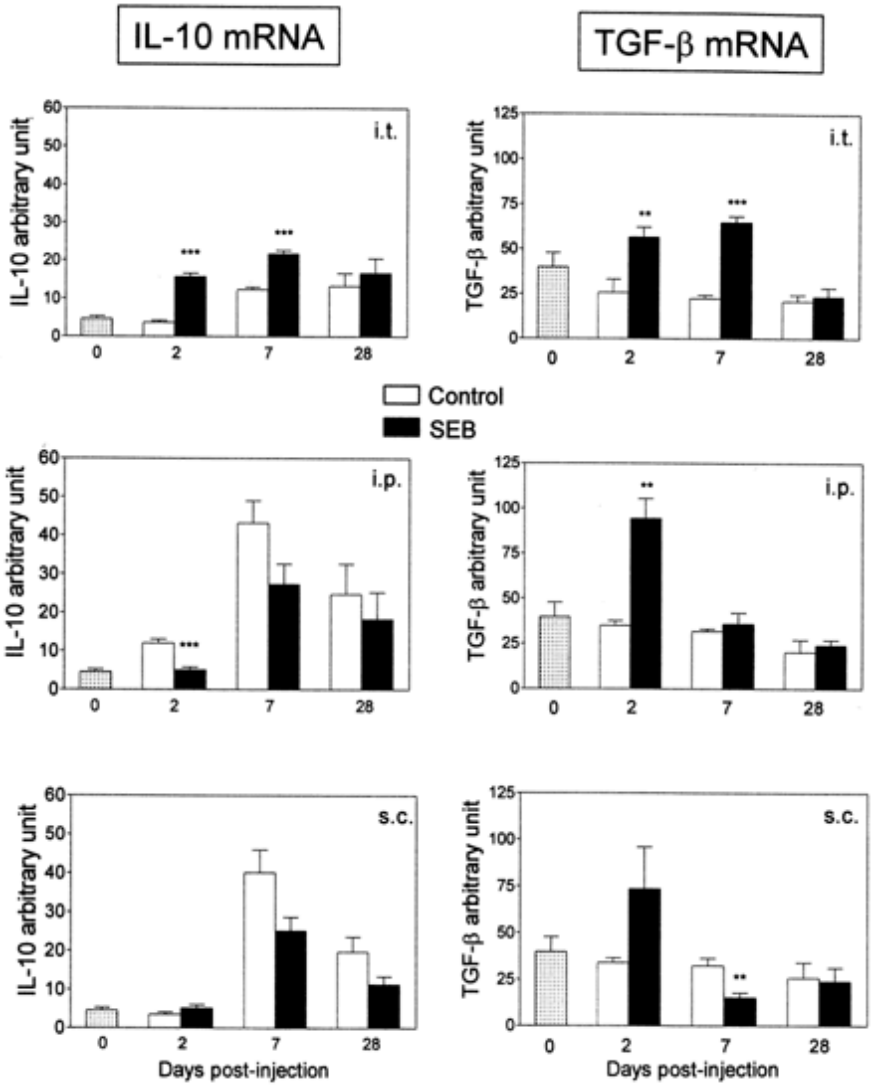


Figure 4
IL-10 (left panels) and TGF-beta (right panels) mRNA expression in splenocytes from saline (control) or SEB-injected mice on days 2, 7, and 28 by the intrathymic

(i.t.), intraperitoneal (i.p.), and subcutaneous (s.c.) routes.

Splenocytes were restimulated for 24 hours with 10 µg/ml of SEB *in vitro.* Results are expressed as arbitrary units and reported as the mean value, ± SEM.

** p < 0.01 and *** p < 0.001 *versus* controls. n = 5 mice/group.

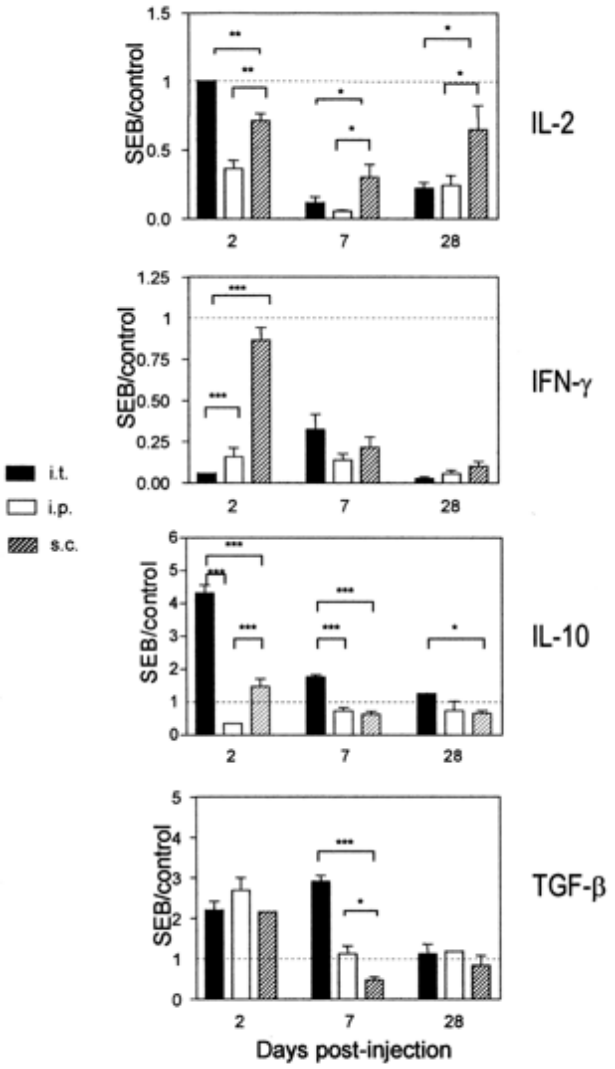


Figure 5
Expression of IL-2, IFN-gamma, IL-10, and TGF-beta mRNAs in total splenocytes on days 2, 7, and 28 after

i.t., i.p., and s.c. SEB-injection. Splenocytes were restimulated for 24 hours with 10 mug/ml of SEB *in vitro*.

Results are expressed as the ratio of mRNA expression in SEB-treated to mRNA expression in control mice. Results are reported as the mean value ± SEM.

* p < 0.05, ** p < 0.01, and *** p < 0.001. n = 5 mice/group.

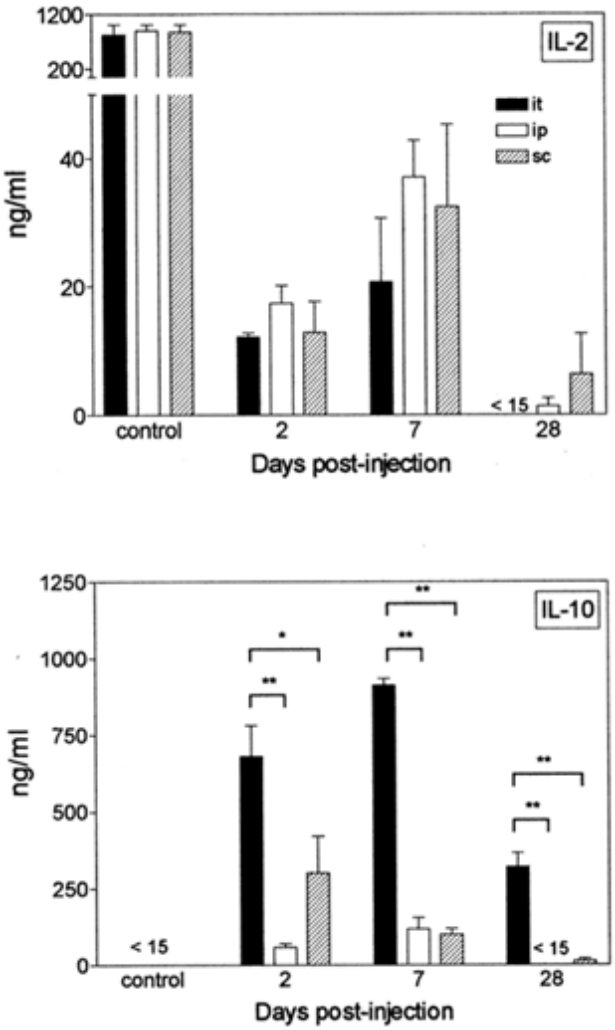


Figure 6

IL-2 and IL-10 release in culture supernatants of total splenocytes from mice injected with SEB by the i.t., i.p., and s.c. routes, determined by ELISA.

Splenocytes were obtained on days 2, 7, and 28 post-injection, then cultured for 24 hours with 10 µg/ml of SEB. Control mice were injected with saline. Results are expressed in ng/ml.

* p < 0.05, ** p < 0.01, and *** p < 0.001 n = 3 mice/group.

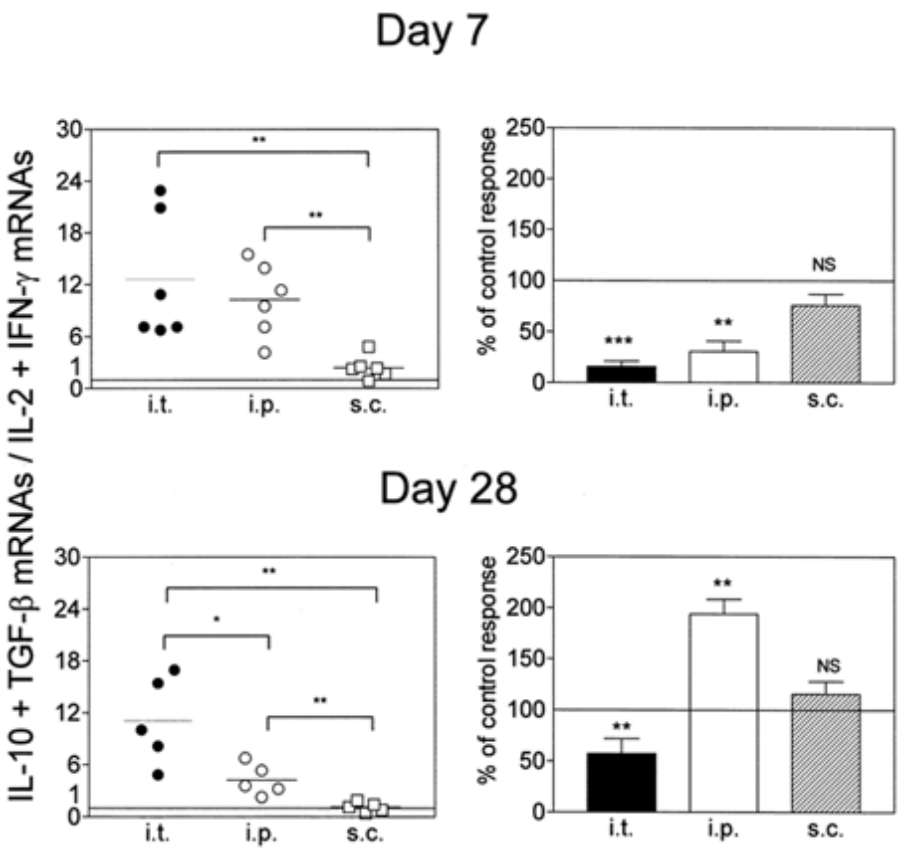


Figure 7
Correlation between the cytokine balance in total splenocytes and the proliferative response *in vitro.*

Left: Ratio of TGF-beta plus IL-10 to IL-2 plus IFN-gamma cytokine mRNAs (IL-10 + TGF-beta mRNAs/IL-2 + IFN-gamma mRNAs) in splenocytes from mice injected with SEB on days 7 (top) and 28 (bottom) after injection by i.t. (closed circles), i.p. (open circles), and s.c. (open squares) routes. Splenocytes were restimulated for 24

hours with 10 µg/ml of SEB *in vitro*. Each point represents one mouse. N = 5 mice/group. The mean value is indicated for each lot.

Right: In vitro proliferative response to 10 µg/ml of SEB of total splenocytes from SEB-treated mice on days 7 (top) and 28 (bottom) after i.t., i.p. or s.c. SEB injections. Results are expressed as the percentage of the proliferative response (in cpm) of SEB-treated mice from the proliferative response (in cpm) of control mice. The mean value with the SEM are reported.

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ *versus* untreated mice.

Table 1
Primers used in quantitative RT-PCR with internal standard (I.S.)

Application	Strand	Sequence	Tm (°C)	Expected size (bp)*
IL-2	sense	5' AACAGCGCACCCACTTCAA 3'	58	442
	antisense	5' TTGAGATGATGCTTTGACA 3'	52	
IFN-gamma	sense	5' TGAACGCTACACACTGCATCTTGG 3'	60	460
	antisense	5' CGACTCCTTTTCCGCTTCCTGAG 3'	62	
beta-actin	sense	5' GGGTCAGAAGGATTCCTATG 3'	58	278
	antisense	5' GGTCTCAAACATGATCTGGG 3'	58	
I.S. IL-2	sense	5' CCAAGCAGGCCACAGAATTATGTTCTGGATTTGACTCA 3'	56	397
	antisense	5' TGAGTCAAATCCAGAACATAATTCTGTGGCCTGCTTGG 3'	56	
I.S. IFN-gamma	sense	5' GACATGAAAATCCTGCAGGGCCATCAGCAACAACATAAGCG 3'	62	413
	antisense	5' CGCTTATGTTGTTGCTGATGGCCCTGGCTCTGCAGGATTTTCATGTC 3'	62	

Table 2
Primers used in LightCycler™ RT-PCR

Application	Strand	Sequence	Tm (°C)	Expected size (bp)
IL-2	sense	5' ACCCTTGCTAATCACTCCTCACAG 3'	58	540
	antisense	5' CTCAGAAAGTCCACCACAGTTGCT 3'	58	
IFN-gamma	sense	5' GTTCTGGGCTTCTCCTCCTG 3'	60	511
	antisense	5' CCGAATCAGCAGCACTCCT 3'	60	
TGF-beta	sense	5' TGGGAAATGCTGCGAGGAGT 3'	58	509
	antisense	5' AATGGCAGGCACAGGTCTTG 3'	58	
IL-10	sense	5' CAACATACTGGCTAACCGACTCC 3'	62	237
	antisense	5' TGCTCCACTGCCTTGCTCTTAT 3'	62	
beta-actin	sense	5' GGGTCAGAAGGATTCCTATG 3'	58	278
	antisense	5' GGTCTCAAACATGATCTGGG 3'	58	
28S	sense	5' CGGGTAAACGGCGGGAGTAA 3'	60	107
	antisense	5' GGTAGGGACAGTGGGAATCT 3'	58	

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real-time TR-PCR, chemokines, Crohn’s disease, ulcerative colitis.

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Cytokine deviation induced by intrathymic injection of staphylococcal enterotoxin B (SEB)

P. Goettelfinger, F. Lecerf, S. Berrih-Aknin, M. German-Fattal
SEB, intrathymic injection, anergy, cytokine deviation, quantitative RT-PCR.

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type 1 diabetes, islets of Langerhans, beta-cells, cytokines, interleukin-1beta, interleukin-1beta receptor type I, nitric oxide, inducible nitric oxide synthase, heat shock protein 70, interferon regulating factor 1.

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