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T cell- and perforin-dependent depletion of B cells *in vivo* by staphylococcal enterotoxin A

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SUMMARY

Bacterial superantigens bind to major histocompatibility complex (MHC) class II and subsequently activate both $CD4^+$ and $CD8^+$ T lymphocytes expressing certain T-cell receptor (TCR)-Vβ chains. In response to superantigen exposure these subsets proliferate, produce large amounts of proinflammatory cytokines and in addition $CD8^+$ cytotoxic T lymphocytes (CTL) are induced. Previous studies *in vitro* have shown that these CTL effectively lyse MHC class II-expressing cells presenting the proper superantigen. However, it is unknown whether superantigens induce a similar response towards MHC class II^+ antigen-presenting cells *in vivo*. In this study we demonstrate that administration of repeated injections of the superantigen staphylococcal enterotoxin A (SEA) to TCR-Vβ3 transgenic mice results in a loss of MHC class II-expressing cells in the spleen. Analysis of different MHC class II^+ subsets revealed a selective depletion of $CD19^+$ B cells, while $F4/80^+$ macrophages increased in number. Depletion of T cells with anti-CD4 or anti-CD8 monoclonal antibody indicated that $CD8^+$ T cells were crucial for SEA-induced cytotoxicity *in vivo*. Repeated injections of SEA to perforin-deficient mice resulted in significantly less B-cell depletion compared with control mice. This suggests that superantigen-activated $CD8^+$ T cells lyse MHC class II^+ antigen-presenting cells in a perforin-dependent manner *in vivo*. It is suggested that this represents a novel bacterial immune escape mechanism, which may particularly impair local humoral immune responses.

INTRODUCTION

The staphylococcal enterotoxins (SE), produced by certain strains of *Staphylococcus aureus*, are some of the most potent mitogens known for T lymphocytes,[1] and have long been recognized as pathogenic in humans.[2] The mitogenic properties of these proteins are linked to their ability to interact with major histocompatibility complex (MHC) class II molecules and subsequently activate both $CD4^+$ and $CD8^+$ T cells expressing the appropriate Vβ-chains in their T-cell receptor (TCR).[3–5] Because the relative number of Vβ genes is limited, a given SE activates a large fraction of T cells, which has rendered SE their denomination as superantigens (SAg).[6] Injection of SE to adult mice leads to a rapid and excessive release of a panel of proinflammatory cytokines.[7,8] The responding T cells up-regulate interleukin-2 (IL-2) receptors and proliferate.[9] Furthermore, $CD8^+$ T cells differentiate into cytotoxic T lymphocytes (CTL).[9,10]

The biological role of SE in the interplay between bacteria and the mammalian immune system is only partially understood. The expression of these immunostimulatory proteins is puzzling since it is important for pathogens to devise strategies allowing them to escape immune surveillance. However, the initial immune response is followed by a long-lasting state of unresponsiveness to subsequent SE challenges. Several distinct immunosuppressive mechanisms have been shown to operate in the unresponsive state, including T-cell deletion,[11,12] anergy[13,14] in responding T cells as well as induction of inhibitory cytokines such as IL-10.[15,16]

Previous studies have demonstrated that injection of SE to mice induces differentiation of responding $CD8^+$ T cells into CTL,[9,10] which are able to lyse MHC class II-expressing target cells *in vitro*. Autologous fresh peripheral blood B cells and monocytes were also shown to be killed efficiently by SE-targeted CTL *in vitro*.[17] In addition, *in vitro* cultures of human peripheral blood mononuclear cells (PBMC) stimulated with SEB resulted either in B-cell activation or in inhibition of immunoglobulin production, depending on the dose of superantigen.[18] The inhibition of antibody response by high doses of SEB was correlated to T-cell-dependent

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Abbreviations: KO, knockout; SAg, superantigen; SDCC, superantigen-dependent cell-mediated cytotoxicity; SE, staphylococcal enterotoxin; Vβ, variable region β-chain.

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cytolysis of B cells.[19] However, no information is available concerning the *in vivo* relevance. In this study we demonstrate that repeated injections of SEA to TCR-Vβ3 transgenic mice resulted in a marked decrease of the number of MHC class II-expressing B cells in spleen and other lymphoid organs. It is postulated that SAg-induced T-cell-mediated B-cell depletion is a bacterial strategy to impair humoral responses and immune recognition.

MATERIALS AND METHODS

Reagents

Monoclonal antibodies (mAb) directed to murine CD4, CD8, TCR-Vβ3, CD19 and I-A^b were purchased from Pharmingen (San Diego, CA). Monoclonal antibody F4/80 detecting murine macrophages was obtained from Serotec (Oxford, UK). Rat hybridomas secreting anti-CD4 (clone GK1.5) and anti-CD8 (clone 2.43) mAb used for *in vivo* depletion of lymphocyte subsets were obtained from the American Type Culture Collection (Rockville, MD). Polyclonal rat IgG was purchased from Jackson Immuno-Research (West Grove, PA).

Animals and treatment

Transgenic C57BL/6 mice expressing a rearranged TCR-Vβ3 gene under the influence of an inserted immunoglobulin heavy-chain enhancer[20] were generously provided by Dr M. Davis (Stanford, CA). Perforin knockout (KO) mice[21] were generously provided by Dr J. Tschopp (Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland). The perforin KO mice were crossed on to TCR-Vβ3^+ background and used for experiments. Recombinant SEA was expressed in *Escherichia coli* and purified to homogeneity as described earlier.[22] Ten micrograms of SEA in phosphate-buffered saline (PBS) with 1% normal syngeneic serum or PBS alone were injected intravenously (i.v.) at 4-day intervals. For depletion of CD4 and CD8 T cells, mice were injected intraperitoneally (i.p.) with 200 μg of anti-CD4 (GK1.5) or anti-CD8 (2.43) or with control rat IgG in PBS 1 and 3 days before the last SEA injection. Depletion of the relevant T-cell subset (>90%) was verified by flow cytometry analysis of spleen cells from individual mice.

Cell lines

The human B-cell lymphoma cell line Raji was cultured in R10-medium [RPMI-1640, Life Technologies LTD, Paisley, UK; supplemented with 10% fetal calf serum, 1 mM glutamine, 1 mM non-essential amino acids (ICN Biomedicals, Costa Mesa, CA), 5×10^{-5} M β-mercaptoethanol and 1 mM sodium pyruvate (Sigma-Aldrich, Irvine, UK)].

Cytotoxicity assay

Cytotoxicity was measured at various effector to target cell ratios in a standard 4-hr ^{51}Cr-release assay.[23] Percent specific cytotoxicity was calculated as

$$100 \times \frac{\text{c.p.m. experimental release} - \text{c.p.m. background release}}{\text{c.p.m. total release} - \text{c.p.m. background release.}}$$

The ^{51}Cr-labelled target cells were used at $2{\cdot}5 \times 10^3$ cells per 0·2 ml R10 medium in microtitre wells. SEA was used at a concentration of 100 ng/ml.

Assay for DNA synthesis

Spleen cells were plated into 96-well microtitre plates using 3×10^5 cells per 0·2 ml R10 medium and were analysed for uptake of [^{3}H]thymidine in the presence or absence of SEA (10 ng/ml). After 1 day of culture, [^{3}H]thymidine was added to the cultures, which were harvested 4 hr later and radioactivity was measured using a liquid scintillation β-counter.

Analysis by flow cytometry

Flow cytometric analysis was performed according to standard settings on a FACSort flow cytometer (Becton Dickinson, Mountain View, CA).

RESULTS

Repeated SEA administrations *in vivo* result in reduced number of MHC-class II-expressing cells

To have a sensitive model to study a possible T-cell-dependent depletion of MHC class II-expressing cells *in vivo*, we used TCR-Vβ3 transgenic mice where all T cells are able to respond to the SAg SEA.[8] Mice were given different numbers of SEA injections with a 4-day interval between injections. Spleens were removed 2 days after the last injection and the cell subsets were analysed by flow cytometry. As previously shown, injection of SEA resulted in an initial expansion of the CD4^+ and CD8^+ T-cell subsets (Fig. 1).[14] However, after repeated treatments, part of the CD4^+ cells were deleted, while the CD8^+ T-cell population continued to expand (Fig. 1). Interestingly, the number of MHC class II (I-A^b)-expressing cells was reduced after one SEA injection compared to untreated control mice (Fig. 1) and continued to decrease with repeated treatments (Fig. 1). The same observation was made when analysing lymph nodes and peripheral blood cells (data not shown). This suggests that the MHC class II-expressing cells in fact had been deleted and excludes a possible migration of these cells to other lymphoid organs.

Selective loss of B cells after SEA treatment

To investigate whether distinct MHC class II-expressing cell subsets exhibit differences in their sensitivity to SEA-dependent depletion, CD19^+ B cells and F4/80^+ macrophages were analysed. These two cell-types are the dominating MHC class II^+ leucocyte subsets in the spleen. TCR-Vβ3 transgenic mice were injected one or three times with SEA and spleen cells were stained for flow cytometry analysis. Analyses of the CD19^+ B cells revealed that the B-cell-compartment was reduced about threefold after three SEA-injections (Fig. 2). In contrast a marked infiltration of F4/80^+ macrophages was observed in the spleen after administration of SEA which persisted with repeated injections (Fig. 2). Thus, this suggested that B cells are sensitive to SEA-induced depletion while macrophages seem to be resistant.

Depletion of CD8^+ T cells restores the number of B cells

In order to investigate which effector cell might be involved in the elimination of B cells, we depleted CD4^+ or CD8^+ T cells *in vivo*. Anti-CD4 or anti-CD8 antibodies were administered prior to the last of three SEA injections to TCR-Vβ3

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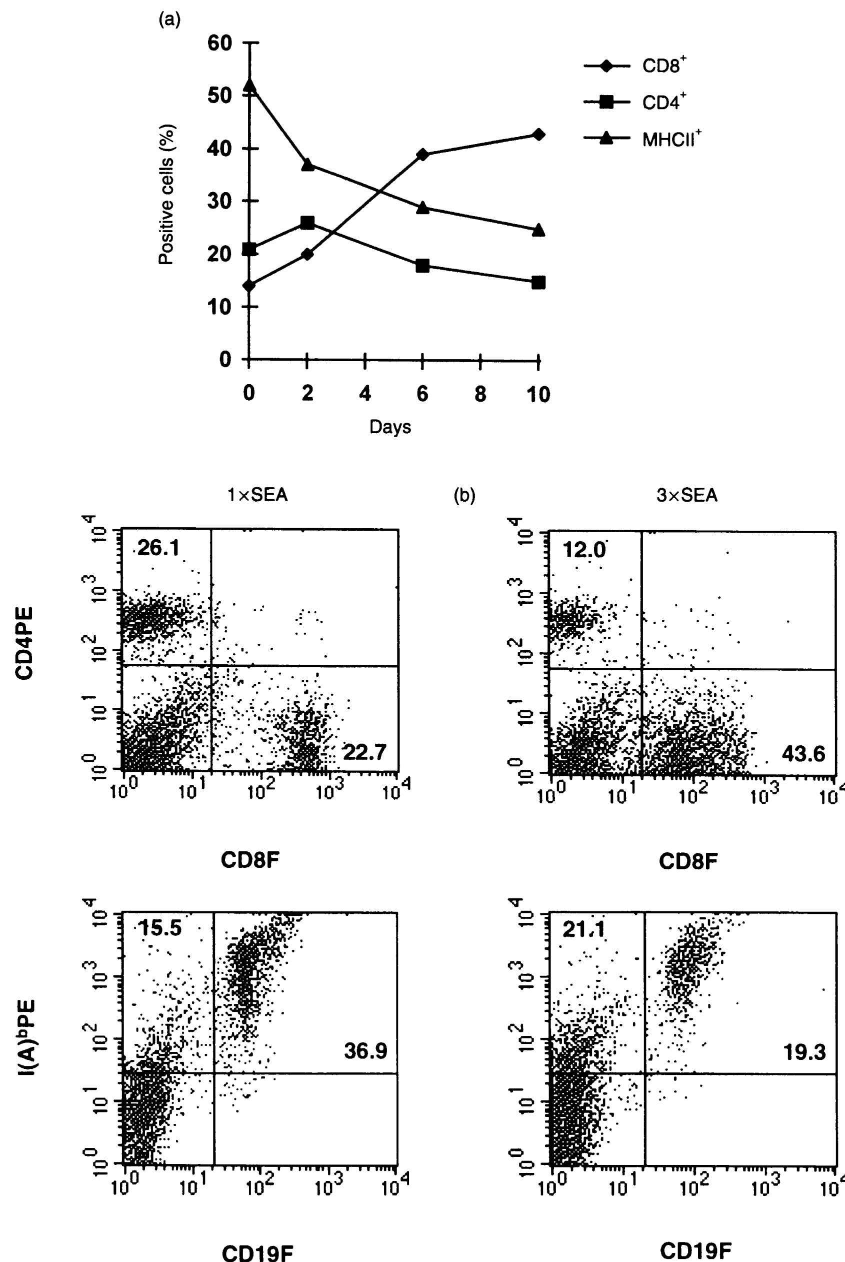

Figure 1. Dynamics of cell-subsets in spleen after repeated SEA injections. TCR-Vβ3$^+$ mice were injected one (day 0), two (day 0 and 4) or three (day 0, 4 and 8) times i.v. with 10 µg SEA at 4-day intervals. Spleens were removed 2 days after the last SEA injection and single-cell suspensions were prepared. These were stained for CD4, CD8 and MHC II (I-A^b) expression and analysed by FACS. (a) The frequencies of positive cells at different time-points are depicted. Mean values from two mice per time-point are shown. (b) Two-colour analysis showing dot-plots of CD4/CD8 and CD19/MHC II (I-A^b) expression on splenocytes from mice treated one or three times with SEA. One out of five similar experiments.

transgenic mice. Depletion of CD4$^+$ T cells had no effect on the number of CD19$^+$ B cells compared to PBS or control-antibody (rat IgG) treatment (Fig. 3). On the contrary, depletion of CD8$^+$ T cells significantly restored the number of B cells ($P<0{\cdot}01$), comparable to the level of untreated mice (Fig. 3). This suggested that CD8$^+$ T cells are responsible for the depletion of MHC-expressing cells *in vivo* as well as *in vitro*.[9,14] Injection of anti-asialoGM1 antibodies which deplete natural killer cells had no effect on the number of B cells (data not shown).

SEA-induced cytotoxicity is severely impaired in perforin-deficient mice

There are two main mechanisms for elimination of target cells by T lymphocytes; one is dependent on the exocytosis of perforin and the other on interaction between the cell-surface receptors Fas and FasL.[24,25] To investigate the role of perforin-dependent cytotoxicity for superantigen-dependent cell-mediated cytotoxicity (SDCC) *in vitro*, mice deficient in perforin (perforin$^{-/-}$)[21] crossed on to TCR-Vβ3 background were used. Perforin$^{-/-}$ ×TCR-Vβ3$^+$ and control TCR-Vβ3$^+$ mice

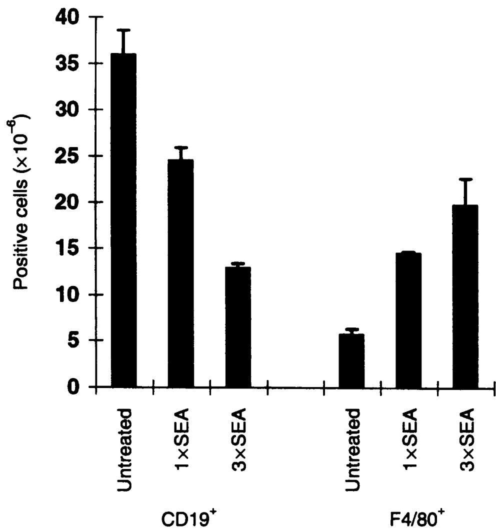

Figure 2. Reduction of $CD19^+$ B cells in spleen after repeated SEA injections. TCR-$V\beta3^+$ mice were injected one or three times i.v. with 10 μg SEA or PBS (untreated control) at 4-day intervals. Spleens were removed 2 days after the last SEA injection and single-cell suspensions were prepared and counted. These were stained for CD19 and F4/80 expression and analysed by FACS. The total number of positive cells is depicted. Mean values ± SD from two individual experiments with two mice/group are shown. One out of five similar experiments.

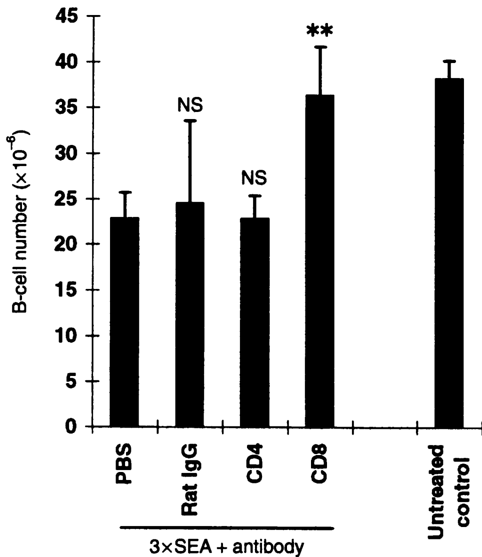

Figure 3. Role of T-cell subsets in the depletion of $CD19^+$ B cells. TCR-$V\beta3^+$ mice were treated three times i.v. with 10 μg SEA or PBS (untreated control) at 4-day intervals. One and three days before the last SEA injection, 200 μg anti-CD4, anti-CD8, or control rat IgG, were injected i.p. Spleens were removed 2 days after the last injection, single-cell suspensions were prepared and counted. The spleen cells were stained for CD19 expression and analysed by FACS. The total number of $CD19^+$ B cells is depicted. Mean values ± SD from four mice/group are shown. The difference from mice not treated with antibody (PBS) was analysed by Student's *t*-test. **, $0{\cdot}01 < P > 0{\cdot}001$; n.s., not significantly different. One out of three similar experiments.

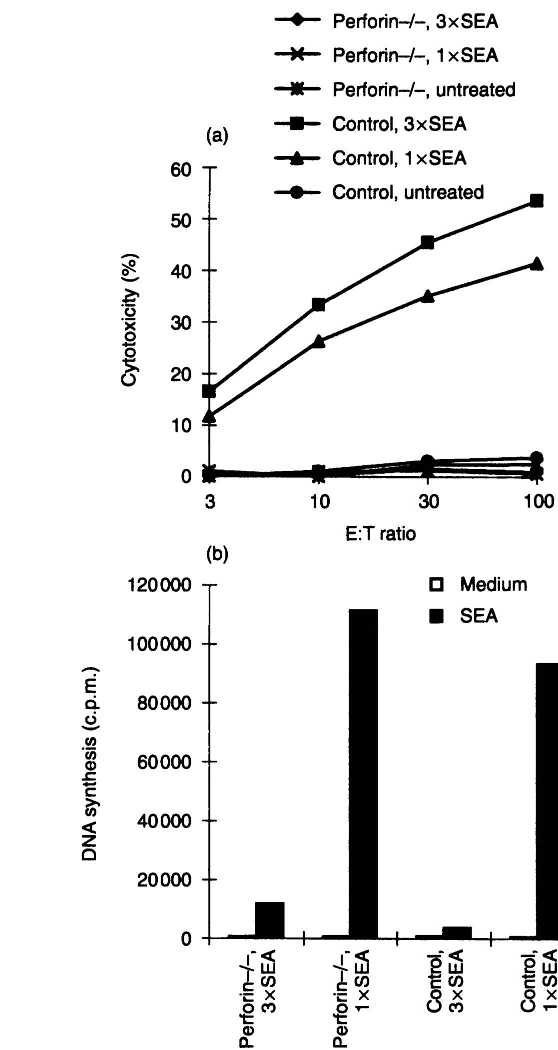

Figure 4. Lack of SDCC in cells from perforin-deficient mice. $Perforin^{-/-}$ x TCR-$V\beta3^+$ and control TCR-$V\beta3^+$ mice were treated one to three times i.v. with 10 μg SEA or PBS (untreated control) with 4-day intervals. Spleens were removed 2 days after the last injection and single-cell supsensions were prepared. (a) Cytotoxicity was measured against SEA-coated (100 ng/ml) Raji cells at different effector to target (E:T) ratios. Standard deviations were routinely less than 10% of the mean value. (b) Proliferation of spleen cells after 1 day of culture in the presence or absence of SEA (10 ng/ml). One out of three similar experiments.

were given different numbers of SEA injections and spleen cells were analysed for cytotoxicity against SEA-coated Raji cells in a standard 4-hr chromium-release assay. Spleen cells from SEA-treated control mice exhibited pronounced cytotoxicity 2 days after injection of one or three doses of SEA (Fig. 4a), while no cytotoxicity could be detected using spleen cells from $perforin^{-/-}$ mice (Fig. 4a). No cytotoxicity was observed in cells from untreated animals (Fig. 4a) nor when target and effector cells were incubated in the absence of SEA (data not shown). Similar proliferative capacity in response to SEA was observed in spleen cells from perforin-deficient and control mice (Fig. 4b), demonstrating that the lack of cytotoxicity was not due to SEA unresponsiveness. Furthermore, flow cytometry analysis of T-cell subsets from $perforin^{-/-}$ mice demonstrated that the T-lymphocyte profile develops in agreement with normal TCR-$V\beta3^+$ mice after repeated SEA treatments (data not shown).

In order to investigate if the observed depletion of B cells *in vivo* is equally perforin-dependent, mice were injected

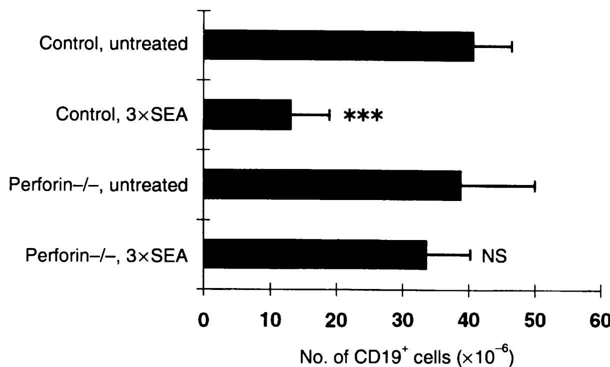

Figure 5. Reduced depletion of $CD19^+$ B cells in perforin-deficient mice. $Perforin^{-/-}$ $\times$TCR-V$\beta3^+$ and control TCR-V$\beta3^+$ mice were treated three times i.v. with 10 µg SEA or PBS (untreated control) at 4-day intervals. Spleens were removed 2 days after the last injection and single-cell suspensions were prepared and counted. These were stained for CD19 expression and analysed by FACS. The total number of $CD19^+$ cells is depicted. Mean values $\pm$ SD from four mice/group are shown. The difference from untreated control mice was analysed by Student's *t*-test. ***, $P<0{\cdot}001$; n.s., not significantly different. One out of six similar experiments.

repeatedly with SEA and analysed for $CD19^+$ B cells in the spleen. Three injections caused a significant loss of $CD19^+$ cells in TCR-V$\beta3^+$ control mice compared with untreated mice ($P<0001$), while only a marginal reduction of B cells was recorded in the $perforin^{-/-}$ $\times$TCR-V$\beta3^+$ mice (Fig. 5). A similar increase of F4/80^+ cells was recorded in the $perforin^{-/-}$ mice after SEA treatment (data not shown) as observed in the controls (Fig. 2). The number of B cells in spleens of perforin-deficient mice were never completely normalized compared to untreated control mice (Fig. 5). This finding suggests that SAg-induced *in vivo* depletion of B cells is predominantly, but not entirely, perforin-dependent.

DISCUSSION

The conservation among different SE to interact with two of the most important molecular families involved in specific immunity, strongly argues that the main role of the SE is related to the immune system. It has been suggested that SE-induced oligoclonal T-cell activation[5] and excessive cytokine release,[7,8] followed by specific T-cell deletions and functional unresponsiveness[11–14] are used by the bacteria to immunocomprise the host.[1] In this study we demonstrate that injection of repeated doses of SEA to TCR-Vβ3 transgenic mice induce a loss of B cells in the spleen and other lymphoid organs, such as lymph nodes and peripheral blood. The mechanism is dependent on $CD8^+$ T cells and involves cytotoxicity mediated by the pore-forming protein perforin.[26] These results suggest that the potent mechanism of SDCC detected against MHC class II-expressing cells *in vitro*[23] also might be operating *in vivo*, where retargeting of CTL to MHC class II^+ antigen-presenting cells could represent an additional strategy of various staphylococcal and streptococcal bacterial strains to evade specific immune recognition.

Elegant experiments in mice containing mutations in Fas (*lpr*) and FasL (*gld*),[27] and KO mice generated deficient in perforin by homologue recombination,[21] have established that these two mechanisms are the predominant pathways in T-cell-mediated cytotoxicity.[24,25] Usage of perforin-deficient mice enabled us to conclude that SDCC *in vitro*, as measured in short-term cytotoxicity assays, was completely perforin dependent. Furthermore, depletion of B cells after SEA treatment *in vivo* was also diminished in perforin KO mice, suggesting that this is the major mechanism for SAg-induced lysis of B cells. However, the number of B cells was not completely restored, indicating that an additional cytotoxic mechanism may contribute to a minor extent. In this line, we observed that cytotoxicity could be detected also in the perforin-deficient mice when prolonging the time of culture (data not shown). Whether this minor activity is dependent on Fas/FasL interactions or release of cytotoxic cytokines, such as tumour necrosis factor, remains to be determined. Interestingly, deletion of SE-responsive $CD4^+$ T cells *in vivo* has been shown to be dependent on Fas/FasL interactions.[28]

While the number of B cells was reduced after repeated SEA injections, the F4/80^+ macrophage-compartment was actually increased. Interactions between lymphocyte function-associated antigen-1 (LFA-1) on T cells and intracellular cell adhesion molecule-1 (ICAM-1) (CD54) on target cells have previously been shown to be crucial for SDCC.[29] However, both cell subsets up-regulated ICAM-1 after SEA treatment. In fact, macrophages were shown to express more CD54 on their cell surfaces compared to B cells (data not shown). In addition, the remaining B cells expressed high levels of MHC class II molecules (Fig. 1b), suggesting that these cells are not deficient in their ability to bind and present the superantigen. Alternatively, the differences observed between B cells and macrophages could be due to intrinsic resistance of macrophages to the perforin-dependent cytotoxicity imposed by $CD8^+$ T cells. In this line, CTL and NK cells appear to be resistant to their own lytic granules[30] through a mechanism that involves expression of a protective molecule.[31] However, both autologous fresh peripheral blood B cells and monocytes have been shown to be killed efficiently by SDCC mediated by human CTL *in vitro*.[17] Whether differentiation of monocytes to macrophages changes their relative susceptibility to perforin remains to be determined. Another possibility might be that the microenvironment in the spleen facilitates the interaction between $CD8^+$ T cells and B cells, resulting in preferential killing of these cells. However, this explanation seems less likely in light of the pronounced macrophage infiltration in spleen after SEA stimulation.

In cultures of human PBMC, staphylococcal toxins have been reported to inhibit B-cell function by decreasing immunoglobulin production.[32] Polyclonal B-cell activation could be achieved only when B cells were cultured in the presence of inactivated T cells (irradiated or mitomycin-treated) or a small number of T cells.[18,33] Further studies revealed that immunoglobulin production was enhanced at low doses of superantigen and inhibited by high doses, which correlated with the level of T-cell-dependent cytolysis of B cells.[19] These observations suggest that a massive activation of T cells may prevent polyclonal B-cell activation. Indeed, in staphylococcal toxic shock syndrome (TSS), where patients are exposed to large concentrations of toxin sufficient to cause serious illness associated with hyper-activation of T cells, the majority of patients fail to develop an antibody response to TSST-1 and other staphylococcal products upon recovery from the illness.[34] In

contrast, in other diseases, such as Kawasaki disease or atopic dermatitis that are probably associated with lower levels of TSST-1 production, B-cell activation occurs.[35,36] Recent studies of anti-SEB immunoglobulin in the general population surprisingly showed the dominance of antibodies towards a single epitope,[37] suggesting the existence of a mechanism that narrows the range of antigen-specific antibodies.

In conclusion, we have demonstrated selective depletion of B cells after repeated superantigen exposure *in vivo* by a process that involves $CD8^+$ T cells and the lytic protein perforin. These results encourage future studies aiming to unravel whether this mechanism leads to an impaired antigen-specific humoral response.

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REFEERENCES

1. Marrack P. & Kappler J. (1990) The staphylococcal enterotoxins and their relatives. *Science* **248,** 705.
2. Kotzin B.L., Leung D.Y., Kappler J. & Marrack P. (1993) Superantigens and their potential role in human disease. *Adv Immunol* **54,** 99.
3. Fraser J.D. (1989) High-affinity binding of staphylococcal enterotoxins A and B to HLA-DR. *Nature* **339,** 221.
4. Fleischer B. & Schrezenmeier H. (1988) T cell stimulation by staphylococcal enterotoxins. Clonally variable response and requirement for major histocompatibility complex class II molecules on accessory or target cells. *J Exp Med* **167,** 1697.
5. Kappler J., Kotzin B., Herron L. *et al.* (1989) Vβ-specific stimulation of human T cells by staphylococcal toxins. *Science* **244,** 811.
6. White J., Herman A., Pullen A.M., Kubo R., Kappler J.W. & Marrack P. (1989) The V beta-specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice. *Cell* **56,** 27.
7. Miethke T., Wahl C., Heeg K., Echtenacher B., Krammer P.H. & Wagner H. (1992) T cell-mediated lethal shock triggered in mice by superantigen staphylococcal B: critical role of tumor necrosis factor. *J Exp Med* **175,** 91.
8. Dohlsten M., Björklund M., Sundstedt A., Hedlund G., Samson D. & Kalland T. (1993) Immunopharmacology of the superantigen staphylococcal enterotoxin A in T cell receptor Vβ3 transgenic mice. *Immunology* **79,** 520.
9. Herrmann T., Bascheri S., Lees R.K. & MacDonald H.R. (1992) *In vivo* responses of $CD4^+$ and $CD8^+$ cells to bacterial superantigens. *Eur J Immunol* **22,** 1935.
10. Hedlund G., Dohlsten M., Petersson C. & Kalland T. (1993) Superantigen-based tumor therapy: *in vivo* activation of cytotoxic T cells. *Cancer Immunol Immunother* **36,** 89.
11. Rellahan B.L., Jones L.A., Kruisbeek A.M., Fry A.M. & Matis L.A. (1990) *In vivo* induction of anergy in peripheral $V\beta8^+$ T cells by staphylococcal enterotoxin B. *J Exp Med* **172,** 1091.
12. MacDonald H.R., Baschieri S. & Lees R.K. (1992) Clonal expansion precedes anergy and death of $V\beta8^+$ peripheral T cells responding to staphylococcal enterotoxin B *in vivo*. *Eur J Immunol* **21,** 1963.
13. Migita K. & Ochi A. (1993) The fate of anergic T cells *in vivo*. *J Immunol* **150,** 763.
14. Sundstedt A., Dohlsten M., Hedlund G., Höidén I., Björklund M. & Kalland T. (1994) Superantigens anergize cytokine-production but not cytotoxicity *in vivo*. *Immunology* **82,** 117.
15. Bean A.G.D., Freiberg R.A., Andrade S., Menon S. & Zlotnik A. (1993) Interleukin 10 protects mice against Staphylococcal Enterotoxin B-induced lethal shock. *Infect Immun* **61,** 4937.
16. Sundstedt A., Höidén 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.
17. Hedlund G., Dohlsten M., Lando P.A. & Kalland T. (1990) Staphylococcal enterotoxins direct and trigger CTL killing of autologous HLA-DR^+ mononuclear leukocytes and freshly prepared leukaemia cells. *Cell Immunol* **129,** 426.
18. Stohl W., Elliott J.E. & Linsley P.S. (1994) Human T cell-dependent B cell differentiation induced by staphylococcal superantigens. *J Immunol* **153,** 117.
19. Stohl W. & Elliott J.E. (1995) Differential human T cell-dependent B cell differentiation induced by staphylococcal superantigens (SAg): regulatory role for SAg-dependent B cell cytolysis. *J Immunol* **155,** 1838.
20. Berg L.J., Pullen A.M., Fazekas de St. Groth B., Mathis D., Benoist C. & Davis M.M. (1989) Antigen/MHC-specific T cells are preferentially exported from the thymus in the presence of their MHC ligand. *Cell* **58,** 1035.
21. Lowin B., Beermann F., Schmidt A. & Tschopp J. (1994) A null mutation in the perforin gene impairs cytolytic T lymphocyte-and natural killer cell-mediated cytotoxicity. *Proc Natl Acad Sci USA* **91,** 11571.
22. Hedlund G., Dohlsten M., Herrmann T. *et al.* (1991) A recombinant C-terminal fragment of staphylococcal enterotoxin A binds to human MHC class II products but does not activate T cells. *J Immunol* **147,** 4082.
23. Dohlsten M., Lando P.A., Hedlund G., Trowsdale J. & Kalland T. (1990) Targeting of human cytotoxic T lymphocytes to MHC class II-expressing cells by staphylococcal enterotoxins. *Immunol* **71,** 96.
24. Kägi D., Vignaux F., Ledermann B. *et al.* (1994) Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. *Science* **265,** 528.
25. Lowin B., Hahne M., Mattmann C. & Tschopp J. (1994) Cytolytic T-cell cytotoxicity is mediated through perforin and Fas lytic pathways. *Nature* **370,** 650.
26. Masson D. & Tschopp J. (1985) Isolation of a lytic pore-forming protein (perforin) from cytolytic T-lymphocytes. *J Biol Chem* **260,** 9069.
27. Nagata S. & Suda T. (1995) Fas and Fas Ligand: lpr and gld mutations. *Immunol Today* **16,** 39.
28. Ettinger R., Panka D.J., Wang J.K.M., Stanger B.Z., Ju S.T. & Marshak-Rothstein A. (1995) Fas Ligand-mediated cytotoxicity is directly responsible for apoptosis of normal $CD4^+$ T cells responding to a bacterial superantigen. *J Immunol* **154,** 4302.
29. Sundstedt A., Höidén I., Hansson J., Hedlund G., Kalland T. & Dohlsten M. (1995) Superantigen-induced anergy in cytotoxic $CD8^+$ T cells. *J Immunol* **154,** 6306.
30. Shinkai Y., Ishikawa H., Hattori M. & Okumura K. (1988) Resistance of mouse cytolytic cells to pore-forming protein-mediated cytolysis. *Eur J Immunol* **18,** 29.
31. Müller C. & Tschopp J. (1994) Resistance of CTL to perforin-mediated lysis: Evidence for a lymphocyte membrane protein interacting with perforin. *J Immunol* **153,** 2470.
32. Moseley A.B. & Houston D.P. (1991) Mechanism of *Staphylococcus aureus* exotoxin A inhibition of Ig production by human B cells. *J Immunol* **146,** 826.
33. Hofer M.F., Newell K., Duke R.C., Schlievert P.M., Freed J.H. & Leung D.Y.M. (1996) Differential effects of staphylococcal toxic shock syndrome toxin-1 on B cell apoptosis. *Proc Natl Acad Sci USA* **93,** 5425.

34. Bohach G.A., Fast D.J., Nelson R.D. & Schlievert P.M. (1990) Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. *Crit Rev Microbiol* **17,** 251.

35. Leung D.Y.M., Meissner H.C., Fulton D.R., Murray D.L., Kotzin B.L. & Schlievert P.M. (1993) Toxic shock syndrome toxin-secreting *Staphylococcus aureus* in Kawasaki syndrome. *Lancet* **342,** 1385.

36. Leung D.Y.M., Harbeck R., Bina P., Hanifin J.M., Reiser R.F. & Sampson H.A. (1993) Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. *J Clin Invest* **92,** 1374.

37. Nishi J.I., Kanekura S., Takei S. *et al.* (1997) B cell epitope mapping of the bacterial superantigen staphylococcal enterotoxin B: the dominant epitope region recognized by intravenous IgG. *J Immunol* **158,** 247.