Immunology 1996 **87** 586–592

Effects of antigen presentation on superantigen-induced apoptosis mediated by Fas/Fas ligand interactions in human T cells

M. BOSHELL, J. McLEOD, L. WALKER, N. HALL, Y. PATEL & D. SANSOM *Bath Institute for Rheumatic Diseases and School of Pharmacy and Pharmacology, University of Bath, Bath, UK*

SUMMARY

Stimulation of T cells with bacterial superantigens has several distinct functional outcomes including proliferation, anergy and apoptosis. At present however, the mechanisms that dictate whether activation, anergy, or apoptosis predominate are unclear. In this study we have investigated the effects of superantigen presentation to mature superantigen-reactive human T-cell lines. Despite expressing major histocompatibility complex (MHC) class II molecules, these lines failed to proliferate in response to superantigen in the absence of antigen-presenting cells (APC) but proliferated when minimal APC were added. In the absence of APC a significant proportion of the T cells underwent apoptosis. This response was rapid, antigen dependent and largely abolished by the addition of cyclosporin A. Interestingly the response was not blocked by the addition of a number of antibodies to cell surface molecules including MHC class II and intracellular adhesion molecule-1. Using both a bioassay and messenger RNA analysis we were able to demonstrate that stimulation of these T cells with superantigen resulted in the induction of Fas-ligand expression on the T cells and furthermore, the ability of these cells to induce apoptosis was inhibited by the addition of blocking Fas antibodies as well as a Fas–Fc fusion protein. These data demonstrate that stimulation of T cells with staphylococcal enterotoxin B induces expression of Fas-ligand resulting in T-cell apoptosis; however, the final outcome of proliferation or apoptosis is determined by the presence of APC.

INTRODUCTION

Following recognition of foreign antigens on the surface of antigen-presenting cells (APC), T lymphocytes become activated and proliferate in order to provide immune effector functions. The co-ordination of both antigen-derived and non-antigen-derived (costimulatory) signals is critical to the activation process and it is apparent that dysregulated activation can result in both antigen-specific unresponsiveness (anergy) and programmed cell death of T cells.[1–3] The regulation of activation versus activation-induced cell death is therefore critical in the development of an effective immune response.

Recently it has become clear that one cell surface molecule which plays a role in the apoptotic death of T cells is the Fas antigen (CD95).[4] This molecule is a 43 000 molecular weight surface glycoprotein with homology to CD40, tumour necrosis factor and nerve growth factor receptors.[5] It has been established that engagement of the Fas antigen by cross-linked monoclonal antibodies[4] and its defined ligand[6,7] induces apoptosis, however, there is also evidence that under certain circumstances Fas may provide costimulatory signals which enhance T-cell responses.[8] Thus it is possible that engagement of Fas antigen by Fas-ligand (Fas-L) may be interpreted as an apoptotic or proliferative signal depending on the context of engagement. This variation in outcome may result from either differences in level of surface Fas expression, the state of differentiation of the responding cell, or the co-ordinated provision of other signals which prevent apoptosis. In this respect, human T cells substantially increase Fas expression following T-cell activation as well as down-regulate survival factors such as bcl-2, the net result being an increased susceptibility to apoptosis in activated T cells.[9,10] This is consistent with a role for Fas in down-regulation of peripheral T-cell responses as seen in murine studies which show that the role of the Fas antigen may be at the level of activation-driven suicide of mature T cells as opposed to a role in thymic deletion.[11] Accordingly, T cells from *lpr* or *gld* mice (which lack functional Fas and Fas-L respectively) are resistant to activation-induced cell death.[12–14] Recently the ligand for Fas has been identified[6] and whilst detailed studies on expression are awaited, evidence based on analysis of mRNA suggests that Fas-L may be restricted to T cells and may participate in the activation-induced cell death of T cells.[15,16] These findings prompt the question as to how apoptosis is prevented since

Received 28 August 1995; revised 28 November 1995; accepted 29 November 1995.

Abbreviations: CsA, cyclosporin A; Fas-L, Fas-ligand; SEB, staphylococcal enterotoxin B.

Correspondence: Dr D. M. Sansom, Bath Institute for Rheumatic Diseases, Trim Bridge, Bath BA1 1HD, UK.

© 1996 Blackwell Science Ltd

both Fas and Fas-L would appear to be up-regulated during T-cell activation.

To address this issue we have investigated the effect of antigen presentation on T-cell responses using the superantigen staphylococcal enterotoxin B (SEB). Several studies in mice have used SEB to study both anergy[17] and activation-induced death,[18] of peripheral T cells which express defined T-cell receptor (TCR) subsets, predominantly $V\beta8^+$. Using this approach it has been demonstrated that following a proliferative phase which results in T-cell expansion, T cells subsequently disappear from the circulation as a result of apoptosis.[18,19] However, the mechanism by which apoptosis occurs is as yet unclear, particularly in respect of the presentation of the antigen. In the present study we have investigated the response of mature human T cells to SEB in the presence or absence of APC. We found that the addition of antigen with transfected APC expressing HLA-DR and B7 molecules was sufficient to result in proliferation of these lines. In contrast, we observed that addition of superantigen to activated T cells directly, in the absence of APC, resulted in apoptosis. Furthermore, our experiments show that the addition of superantigen causes an up-regulation of Fas-L and that this is responsible for the antigen-induced death. Thus T cells may either proliferate or undergo apoptosis in response to a superantigen depending on the nature of antigen presentation.

MATERIALS AND METHODS

T-cell lines

SEB-reactive T-cell lines were established from peripheral blood of healthy donors. Mononuclear cells were isolated by density gradient centrifugation incubated with 1 μg/ml SEB, after 4 days the cells were given fresh medium (complete medium: RPMI-1640 + 10% fetal calf serum (FCS), penicillin and streptomycin) and interleukin-2 (IL-2; 100 U/ml) as required. After day 14, lines were restimulated with superantigen 1 μg/ml presented by fixed CHO-DR4-B7 transfectants and following a further 5 days in culture purified on Ficoll to remove dead cells. Lines were then used in proliferation and apoptosis assays.

SEB-induced death

SEB was added to 1×10^6 cells at the concentrations and times shown and the cells were incubated at 37° in complete medium in 24-well plates. After treatment, cells were fixed in 70% ethanol in phosphate-buffered saline (PBS) and stained with propidium iodide for analysis of DNA content by flow cytometry as described by Darzynkiewicz *et al.*[20] Briefly, following treatment with SEB 5×10^5 cells were fixed for 1 hr in ice cold 70% ethanol/PBS. Cells were then pelleted and resuspended in 500 μl of PBS containing RNAase (20 μg/ml) and incubated at 37° for 1 hr. Propidium iodide was added to 1 μg/ml and the cells were analysed by flow cytometry for the presence of a sub-G_0 DNA peak. Blocking antibodies were added at the start of the cultures at a final concentration of 10 μg/ml. Cyclosporin A (CsA) and ionomycin were used at the concentrations indicated.

T-cell proliferation

Fifty thousand T-cell blasts were stimulated by the addition of SEB at the concentrations shown, in the presence of transfected DR4, DR4-B7 or control CHO cells. Chinese hamster ovary cells expressing human leucocyte antigen (HLA) DR4 molecules and coexpressing the costimulatory molecule B7 were generated as described previously.[21] CHO cells were fixed for 2 min in 0·025% glutaraldehyde, then washed extensively in complete medium before being added (1×10^4/well) in 96-well plates. After 72 hr of culture, wells were pulsed for 16 hr with 0·5 μCi [^{3}H]thymidine, harvested onto glass fibre filters and counted by scintillation counting.

Monoclonal antibodies

The following antibodies were used as either staining reagents or blocking reagents at 10 μg/ml: OKT11 (anti-CD2), UCHT1 (anti-CD3), HB8784 (anti-CD25), 9·3 (anti-CD28), L243 (anti HLA-DR), BB1 (anti-CD80), CH11 (anti-Fas CD95), TS2/9 (anti-CD58), 15·2 (anti-CD54) and 38 (anti-LFA-1). The anti-Fas antibody M3 was a generous gift from Dr D. Lynch (Immunex, Seattle) and was used at 3 μg/ml. Staining was detected by addition of fluorescein isothiocyanate (FITC)-conjugated anti-mouse polyvalent immunoglobulins (Sigma) and analysed by flow cytometry.

Fas-ligand bioassay

Fas-sensitive Jurkat cells (1×10^7) were labelled with 1 mCi Na ^{51}Cr for 2 hr at 37° in complete medium. Cells were washed extensively in complete medium and used at 5×10^4/well in a 96-well plate. Fas-L expressing T cells were incubated with the Jurkat cells at the ratios shown in the presence or absence of SEB at 1 μg/ml for 16 hr, with either a Fas-specific monoclonal antibody or Fas–Fc protein as inhibitor. Inhibitors were used at the concentrations shown in Fig. legends. The % specific killing was calculated as observed release − spontaneous release/maximum spontaneous release × 100. Maximum release was measured by adding 1% nonidet P.40 to the cultures.

Reverse transcription–polymerase chain reaction for Fas-ligand

Fas-ligand messenger RNA was detected using a reverse transcriptase–polymerase chain reaction (RT-PCR) assay. Briefly, RNA was isolated by the method of Chomczynski & Sachhi.[22] Five hundred nanograms of RNA was reverse transcribed for 1 hr. Subsequently, 2 μl of the resulting cDNA was subjected to PCR using the primers 5′ GTTTGCTG-GGGCTGGCCTGACT 3′ and 5′ GGAAAGAATCCCAAA-GTGCTTC 3′ as the forward and reverse primers respectively. Thirty cycles were carried out at 94° (1 min), 55° (1·5 min) and 72° (1·5 min). As a control for reverse transcription and PCR, the following primers 5′ GGTGAAGGTCG-GAGTCAACGG 3′ and 5′ GGTCATGAGTCCTTCCAC-GAT 3′ were used to amplify the GAPDH gene. Finally 10% of the reaction product was analysed on a 1% agarose gel and visualized by ethidium bromide fluorescence.

RESULTS

Class II positive T cells require APC for proliferation

During studies of the accessory molecule requirements of APC in activating T cells, we observed that superantigen-reactive

Table 1. Phenotypic analysis of T cells from two representative SEB-reactive cell lines. Cells (2×10^5/antibody) were removed from culture and washed in PBS. Cells were resuspended in the antibodies shown above for 45 min at 0°, washed in PBS and detected with anti-mouse FITC. Cells were analysed for FITC fluorescence using a Becton Dickinson FACStar Plus using a 60 mW 488 nm Argon laser. Cells were analysed for mean fluorescence intensity (MFI) and percentage positivity (% cells) above a region set at the 95 centile of the negative control

	Line 1		Line 2	
Antibody	MFI	% cells	MFI	% cells
NEG	2·3	6·7	3·3	2·7
CD2	42·4	99·8	112·9	99·4
CD3	56·9	99·6	36·6	97·0
CD4	24·7	89·3	35·6	88·9
CD25	20·1	27·0	101·2	97·2
CD28	12·0	73·9	15·5	46·9
HLA-DR	26·3	37·0	63·7	71·0
CD95	25·9	97·6	28·3	98·1

T-cell lines had a high dependence on APC in order to proliferate. To study these effects further, T cell lines were generated which were reactive to SEB and which possessed the phenotypes of activated T cells (shown in Table 1). These lines were then restimulated with SEB in the presence or absence of transfectants expressing class II MHC molecules. Despite expressing HLA-DR molecules, the T-cell lines did not proliferate to superantigens added to the culture in the presence of control CHO cells. However the addition of both HLA-DR and particularly HLA-DR-B7 expressing transfectants restored the response to SEB indicating that the cells were both antigen-specific and capable of a proliferative response to antigen (Fig. 1). This experiment suggested that whilst presentation through HLA-DR on transfectants was capable of stimulating

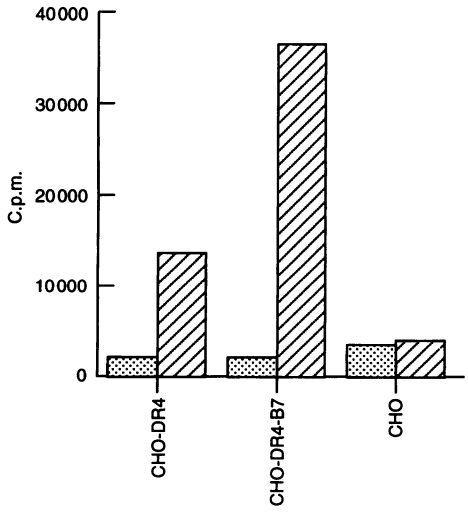

Figure 1. Proliferative response of T-cell lines to rechallenge with SEB. T cells (5×10^4) were stimulated by the addition of 1 μg/ml SEB (hatched bars) or no antigen (dotted bars), in the presence of 1×10^4 transfected CHO cells as APC. Responses are shown as c.p.m. [^{3}H]-thymidine incorporation. Data are representative of five experiments.

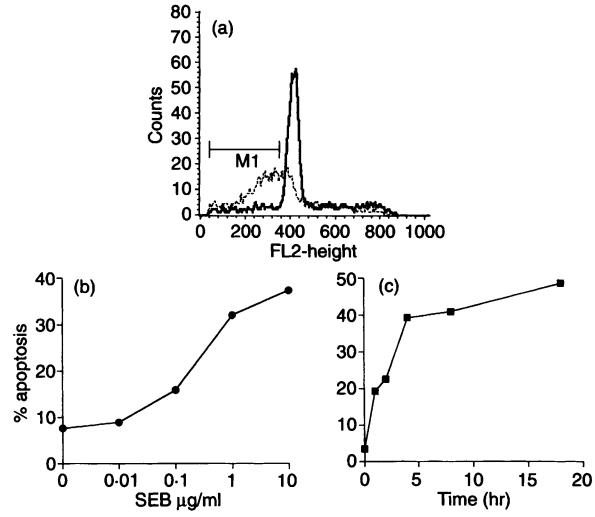

Figure 2. Induction of apoptosis by superantigen. (a) DNA histograms of T cells treated in the absence (solid line) or presence (dashed line) of 1 μg/ml SEB for 16 hr; percentage apoptosis was measured as accumulation of DNA in the sub-G_0 region (M1). (b) Dose response of T cells to addition of SEB for 16 hr; the percentage apoptotic cells accumulated in the region M1 were then measured as above. (c) Kinetics of apoptosis induced by SEB; 1 μg/ml SEB was incubated with the T cells, samples removed for assay at the time indicated and the assay stopped by fixation in ethanol. DNA was assayed for the appearance of sub-G_0 DNA as above. Data are representative of six experiments.

proliferation, the engagement of superantigen directly by T cells did not result in proliferation, despite the expression HLA-DR molecules. We therefore investigated further the effects on T cells of such non-prolifertive encounters with superantigen.

Superantigen induces rapid apoptosis in reactive T-cell lines

Since it had previously been observed in mice that superantigens could induce an array of responses including anergy and apoptosis in T cells, we investigated whether our T-cell lines displayed any of these features during non-proliferative encounters with SEB. Using flow cytometric analysis of the DNA content of the T cells, we observed a striking increase in cells whose nuclei showed signs of apoptosis as a result of the addition of SEB. Apoptotic features were seen as an accumulation of DNA staining in a sub-G_0-G_1 region of the DNA histogram, reflecting condensation and degradation of the nuclear DNA (Fig. 2a). The addition of SEB to T cells resulted in a dose-dependent response leading to apoptosis of up to 40–50% of the T cells at 10 μg/ml (Fig. 2b). Furthermore, an investigation of the kinetics of this response revealed that nuclear changes were observed after as little as 1 hr following addition of antigen reaching almost maximal response after as little as 4 hr (Fig. 2c). These features were highly consistent with an apoptotic response and we concluded that the addition of superantigen directly to the T-cell cultures caused the rapid induction of apoptosis.

Apoptosis depends on a calcium-mediated signal

Given that SEB is known to induce activation signals via engagement of the T-cell receptor we wished to establish

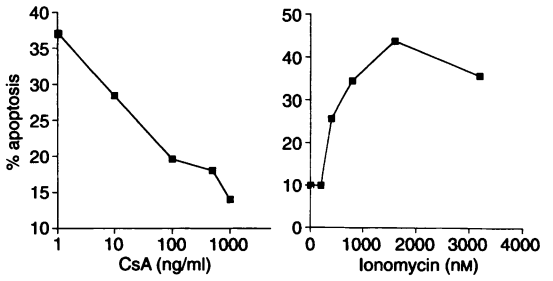

Figure 3. (a) Effect of CsA on SEB-induced apoptosis. CsA was added to T cell cultures 30 min prior to SEB (1 μg/ml) and the cells were incubated for 16 hr. (b) The effect of ionomycin on the induction of T-cell apoptosis. Apoptotic DNA was measured as in Fig. 2. Data are representative of two experiments.

whether apoptosis of our T cells involved this route. To further study the mechanism involved we investigated the effect of agents which either inhibit or mimic signalling via the TCR receptor such as CsA and ionomycin. Using this approach it could readily be seen that apoptosis induced by SEB was almost completely sensitive to inhibition by cyclosporin (Fig. 3a). This clearly indicated a role for a calcium/calcineurin-mediated signal in the induction of apoptosis by SEB supporting the involvement of the TCR in the induction of cell death. Consistent with this finding was the observation that SEB-induced apoptosis could be mimicked by the addition of the calcium ionophore ionomycin (Fig. 3b). Both of these experiments clearly suggested that an intracellular calcium signal was important in the induction of apoptosis by SEB.

Apoptosis is not inhibited blocking cell adhesion

In order to analyse whether T-cell contact was an important feature of apoptosis in response to SEB, we performed antibody blocking experiments in an attempt to prevent apoptosis (Fig. 4). Suprisingly, of the antibodies tested, none were particularly effective in inhibiting cell death. The most effective were those against adhesion molecules LFA-1 (38·1) and LFA-3 (TS2/9). This suggested however, only a limited involvement of cell contact in the induction of apoptosis in this system. Perhaps most suprisingly an antibody against the HLA-DR molecule was also ineffective indicating that presentation of superantigen through HLA-DR on T cells might not be necessary in to induce apoptosis. The above experiments led us to conclude that the response to SEB did not necessarily involve antigen presentation to T cells but possibly activation of T cells via a direct interaction of the SEB molecule with the TCR resulting in a calcium signal.

Apoptosis is mediated by a Fas/Fas-ligand interaction

Since ligation of the Fas antigen has been shown to induce apoptosis in T cells, we sought to investigate the role of Fas and Fas-L in the death of our T-cell lines. Accordingly we determined whether Fas-L might be present or induced on the surface of our activated T cells as a result of superantigen stimulation. Using a sensitive ^{51}Cr-release assay, Jurkat cells were labelled and used as Fas-sensitive target cells to measure Fas ligand on activated T cells. Chromium release was then

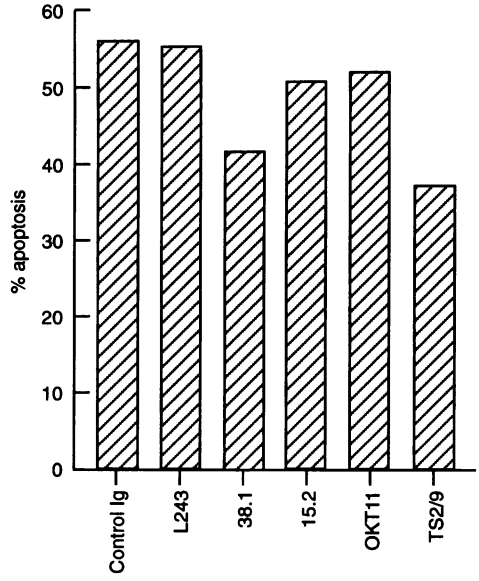

Figure 4. Effect of antibody blocking on SEB-induced apoptosis. Monoclonal antibodies to the following surface molecules: HLA-DR (L243), lymphocyte function-associated antigen-3 (LFA-1) (38·1), intracellular adhesion molecule-1 (ICAM-1) (15·2), CD2 (OKT11), LFA-3 (TS2/9), were added (10 μg/ml) at the start of culture and incubated for 16 hr in the presence of SEB. Apoptosis was measured as given in Figu.2. Data are representative of two experiments.

used to measure the degree of killing of Jurkat cells by the T cell lines and the effect of a blocking anti-Fas antibody and a recombinant Fas–Fc protein used to measure the specificity of cell death (Fig. 5). This experiment clearly showed the presence of Fas-L induced on the T cell blasts as a result of SEB stimulation (Fig. 5a), as well as a degree of basal expression in some lines (Fig. 5b) which was further increased on SEB stimulation. In addition, the death induced by Fas-L expression was clearly inhibited by the addition of the anti-Fas and Fas-L reagents, confirming that the cytotoxicity was due to engagement of Fas by the activated T cells. We therefore concluded that SEB activation resulted in the up-regulation of Fas-L on SEB blasts which could then induce cell death in Fas-positive

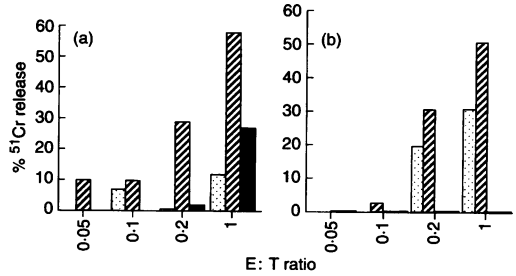

Figure 5. Fas-ligand expression on SEB-activated T-cell blasts. Two independent T-cell lines (panels a and b) were assessed for the ability to induce apoptosis in ^{51}Cr-labelled Fas-sensitive Jurkat cells at various effector to target ratios. Labelled Jurkat cells were co-incubated with T-cell blasts in the presence (hatched bars) or absence (dotted bars) of SEB. The blocking effects of 3 μg/ml of an anti-Fas antibody, M3 (black bars) or 1/10 dilution a recombinant Fas–Fc protein (grey bars, panel b only) were measured. Data are representative of four experiments.

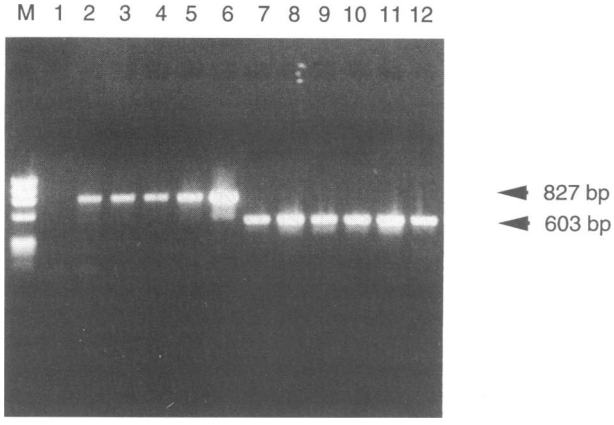

Figure 6. Induction of Fas-L mRNA in SEB-reactive T-cell blasts. RNA was extracted and subjected to RT–PCR for Fas-L (lanes 1–5) and GAPDH (lanes 7–11). T-cell blasts 5×10^6, were stimulated with SEB (1 μg/ml) for 0 hr (lanes 1 and 7), 1 hr (lanes 2 and 8), 2 hr (lanes 3 and 9), 3 hr (lanes 4 and 10) and 4 hr (lane 5 and 11). Plasmid control cDNAs are shown in lanes 6 and 12. Molecular weight standards ($\phi \times 174/Hae$III) are marked M.

target cells. In addition to these findings we also performed FACS analysis of DNA on the T-cell blasts which also underwent apoptosis in response to SEB. This experiment also confirmed that the appearance of a sub-G_0 DNA peak was prevented by the addition of the M3 antibody (data not shown).

Finally to establish that the kinetics of Fas-L up-regulation were consistent with the kinetics of apoptosis induction, SEB blasts were stimulated with SEB and Fas-L mRNA induction measured by RT-PCR. This showed (Fig. 6) that following stimulation with SEB the messenger RNA for Fas-L was detectable within 1 hr of stimulation, reaching a maximum at approximately 4 hr. This data confirmed the ability of T cell blasts to rapidly upregulate Fas-L mRNA, consistent with the rapid expression of this molecule in T cells undergoing apoptosis.

DISCUSSION

In order to generate an effective immune response, T lymphocytes require the co-ordinated presentation of antigen in association with costimulatory signals. Engagement of the T-cell receptor in the absence of costimulation leads to 'negative' outcomes which may include anergy and apoptosis, particularly in activated cells. However, at present it is unclear which molecules are involved in the induction of these negative states or how they are regulated. Interestingly in many of the systems in which T-cell anergy results, the same stimuli can also cause considerable apoptosis,[2,23,24] this, coupled with the observation that Fas-defective mice have altered tolerance mechanisms,[25] suggests that here may be an overlap between anergy and apoptosis. In this study we have investigated the functional outcomes following presentation of the superantigen SEB to activated human T cells in the presence and absence of APC. Predictably, superantigen-primed T cells were capable of vigorous proliferative responses to the superantigen when presented by minimal APC expressing HLA-DR or HLA-DR-B7. However, in contrast, the addition of the superantigen alone induced T-cell apoptosis. This result was somewhat suprising in that the human T-cell lines used also expressed HLA-DR molecules yet were incapable of mounting a proliferative response to antigen. Thus the resultant apoptosis appeared to be a feature of T-cell antigen presentation.

The ability of T cells to present antigen to other T cells has been the source of controversy over a number of years. In many instances MHC class II positive T-cell lines become tolerant following the addition of antigenic peptide.[26–29] However, in other cases T cells appear to be useful APC capable of expressing costimulatory molecules[30] and stimulating allo-responses.[31,32] In general, however, stimulation of T cells with anti-CD3 antibodies, peptides and superantigens frequently results in anergy and apoptosis.[2,16,23,24,29,33] Recently it has become clear that in these systems the above stimuli are sufficient to induce the Fas-L expression on T cells, an event which is prevented by the addition of CsA[34] in what appears to be the major mechanism for activation-induced cell death in T cells.[16,35] Clearly these results are consistent with our findings of SEB-induced Fas-L expression.

One major question prompted by our studies is why do T cells undergo apoptosis in response to superantigen alone but proliferate in response to transfected DR4 and DR4-B7 APC. Given the strong response to the DR4-B7 transfectants, one possibility is that B7-CD28 interactions provide a costimulus which overrides the Fas death signal allowing proliferation. This is attractive considering the role CD28 plays in preventing the induction of anergy[36] and the observation that CD28 can up-regulate the Bcl-X_L gene which is protective from Fas-induced apoptosis.[37] Since we observe some proliferative recovery by the addition of DR4-positive transfectants alone, this may not be the sole mechanism. An additional possibility is that the CHO-DR or CHO-DR-B7 transfectants provide an alternative, possibly more efficient target for antigen presentation to T cells. The levels of major histocompatibility (MHC) class II on these cells are considerably higher than that on T cells and they may bind more free superantigen thereby limiting its effects on the T cells. Thus transfected APC may provide a Fas-L^- presenting cell which can induce T-cell proliferation instead of apoptosis. The implications of this finding are that activated, Fas-sensitive T cells may proliferate or undergo apoptosis depending on the nature of the APC they encounter. It will therefore be of considerable interest to determine the expression of Fas-L on professional APC such as dendritic cells and monocytes. Clearly, since human T cells are equipped to present antigen by expression of MHC class II molecules, the presentation of antigen by T cells may serve as a mechanism of down-regulating T-cell responses as previously suggested.[38]

Evidence from our antibody blocking studies suggested a suprising lack of involvement of HLA-DR expressed by T cells in the apoptotic response. This may be explained by the fact that SEB has previously been shown to be capable of interacting directly with the TCR, in the absence of MHC molecules.[39] In these studies this interaction was suggested to be capable of inducing anergy in T cells, by delivery of a calcium signal. Clearly the concept of SEB interacting directly with the TCR and causing a calcium signal is consistent with our findings and this mechanism may be significant in mice where T cells undergo apoptosis in response to superantigen but do not express MHC class II molecules.[19,33] In addition, activation of the TCR has previously been shown to increase

adhesion via lymphocyte function-associated (LFA-1) molecules.[40] Thus the addition of superantigen may serve to generate a signal capable of inducing expression of Fas-L as well as promoting T cell aggregation. This would then explain the partial sensitivity of apoptosis to anti-adhesion molecule antibodies but not to HLA-DR antibodies. Whilst SEB binding to T-cell MHC is not precluded in this model, inhibition of MHC molecules alone would not be sufficient to prevent apoptosis.

Our finding that ionomycin also induces apoptosis may also be explained by the fact that activated T-cell blasts can proliferate in response to ionomycin alone following antigenic stimulation. This presumably reflects synergy between the calcium signal and pre-existing factors generated as a result of full activation, sufficient to allow up-regulation of Fas-L. However, in contrast with proliferative outcomes, the induction of Fas-L leads to death in Fas-sensitive T-cell lines.

Given that it is clear that activated T cells up-regulate Fas thereby increasing their susceptibility to apoptosis, and express Fas ligand, regulation of Fas-induced death would appear critical in order that effective T cell responses could be established. Such protection may occur at several levels. Firstly, the level of Fas in resting T cells may be insufficient to cause apoptosis thereby necessitating up-regulation before apoptosis can be induced. Secondly, whilst Fas expression may be sufficient there may be other factors required (possibily associated with T-cell differentiation/maturation) to initiate the death outcome in response to Fas engagement and evidence for such an effect has been reported.[9,41] In line with this possibility we have also observed several T-cell lines which are clearly resistant to both SEB and anti-Fas induced death despite expression of the Fas antigen. Thirdly there may be other factors which allow rescue from Fas-L up-regulation or Fas-induced apoptosis. In this respect expression of costimulatory molecules by T cells such as B7[30] may prove important. Recent data from Yang *et al.* has recently shown that both dexamethasone and retinoic acid can block CD3-induced Fas-L up-regulation, thereby indicating that some signals are capable of suppressing Fas-L expression. Clearly all of the above mechanisms may provide effective escape from Fas-driven activation induced cell death and further work is now required to understand the response of T cells to encounters with both antigen and Fas-L.

ACKNOWLEDGMENTS

We are extremely grateful to Dr D. Lynch (Immunex, Seattle), and Dr J.-Y. Bonnefoy (Glaxo IMB Geneva), for the gifts of M3 and Fas–Fc respectively. DS and MB are funded by the Arthritis and Rheumatism Council, LW and YP are funded by the Wellcome Trust and JM by the Oliver Bird Fund.

REFERENCES

1. Schwartz R.H. (1990) A cell culture model for T lymphocyte clonal anergy. *Science* **248,** 1349.
2. Russell J.H., Rush B., Abrahams S.I. & Wang R. (1992) Sensitivity to anti-CD3-stimulated suicide is independent of functional phenotype. *Eur J Immunol* **22,** 1655.
3. Groux H., Monte D., Plouvier B., Capron A. & Ameisen J.-C. (1993) CD3-mediated apoptosis of human medullary thymocytes and activated peripheral T cells: respective roles of interleukin-1, interleukin-2, interferonγ and accessory cells. *Eur J Immunol* **23,** 1623.
4. Itoh N., Yonehara S., Ishii A. *et al.* (1991) The polypeptide encoded by the cDNA for the cell surface antigen Fas can mediate apoptosis. *Cell* **66,** 233.
5. Smith C.A., Farrah T. & Goodwin R.G (1994) The TNF receptor superfamily of cellular and viral proteins: activation, costimulation and death. *Cell* **76,** 959.
6. Suda T., Takahashi T., Golstein P. & Nagata S. (1994) Molecular cloning and expression of the Fas ligand, a novel member of the Tumour Necrosis Factor family. *Cell* **75,** 1169.
7. Tanaka M., Suda T., Takahashi T. & Nagata S. (1995) Expression of the functional soluble form of human Fas ligand in activated lymphocytes. *EMBO J* **14,** 1129.
8. Alderson M.R., Armitage R.J., Marakovsky E. *et al.* (1994) Fas tranduces activation signals in normal human T lymphocytes. *J Exp Med* **178,** 2231.
9. Miyawaki T., Uehara T., Nibu R. *et al.* (1992) Differential expression of apoptosis-related Fas antigen on lymphocyte subpopulations in human peripheral blood. *J Immunol* **149,** 3753.
10. Salmon M., Pilling D., Borthwick N.J. *et al.* (1994) The progressive differentiation of primed T cells is associated with an increasing susceptibility to apoptosis. *Eur J Immunol* **24,** 892.
11. Sidman C., Marshall N.D. & von Boehmer H. (1992) Transgenic T cell receptor interactions in the lymphoproliferative and autoimmune syndromes of the *lpr* and *gld* mutant mice. *Eur J Immunol* **22,** 499.
12. Russell J.H., Rush B., Weaver C. & Wang R. (1993) Mature T cells of autoimmune lpr/lpr mice have a defect in antigen-stimulated suicide. *Proc Natl Acad Sci USA* **90,** 4409.
13. Gillette-Ferguson I. & Sidman C.L. (1994) A specific intercellular pathway of apoptotic cell death is defective in the mature peripheral T cells of *lpr* and *gld* mice. *Eur J Immunol* **24,** 1181.
14. Watanabe-Fukunaga R., Brannan C.I., Copeland N.G., Jenkins N.A. & Nagata S. (1992) Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* **356,** 314.
15. Alderson M.R., Tough T., Davis-Smith T. *et al.* (1995) Fas ligand mediates activation-induced cell death in human T lymphocytes. *J Exp Med* **181,** 71.
16. Dhein J., Walczak H., Baumler C., Debatin K.-M. & Krammer P.H. (1995) Autocrine T cell suicide mediated by APO-1/(Fas/CD95). *Nature* **373,** 438.
17. Kawabe Y. & Ochi A. (1990) Selective anergy of Vβ8$^+$, CD4$^+$ T cells in staphylococcal enterotoxin B primed mice. *J Exp Med* **172,** 1065.
18. MacDonald H.R, Bashieri S. & Lees R.K (1991) Clonal expansion precedes anergy and death of Vβ8$^+$ positive peripheral T cells responding to staphylococcal enterotoxin B *in vivo*. *Eur J Immunol* **21,** 1963.
19. Kawabe Y. & Ochi A. (1991) Programmed cell death and extrathymic reduction of Vβ8 CD4$^+$ T cells in mice tolerant to *Staphylococcus aureus* enterotoxin B. *Nature* **349,** 245.
20. Darzynkiewicz Z., Bruno S., Delbino G. *et al.* (1992) Features of apoptotic cells measured by flow cytometry. *Cytometry* **13,** 795.
21. Sansom D.M., Wilson A., Boshell M., Lewis J. & Hall N.D (1993) B7/CD28 but not LFA-3 CD2 interactions can provide third party costimulation for human T cell activation. *Immunology* **80,** 242.
22. Chomczynski P. & Sacchi N. (1987) Single step method of RNA extraction by guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162,** 156.
23. Nicolle M.W., Nag B., Sharma S.D. *et al.* (1994) Specific tolerance to an acetyl choline receptor epitope induced *in vitro* in myasthenia gravis CD4+ lymphocytes by soluble major histocompatibility complex class II-peptide complexes. *J Clin Invest* **93,** 1361.

24. Jenkins M.K., Chen C., Jung G., Mueller D.L. & Schwartz R.H. (1990) Inhibition of antigen specific proliferation of type 1 murine T cell clones after stimulation with immobilized anti CD3 monoclonal antibody. *J Immunol* **144,** 16.
25. Zhou T., Bluethmann H., Zhang J., Edwards III K. & Mountz J.D. (1992) Defective maintenance of T cell tolerance to a superantigen in MRL *lpr/lpr* mice. *J Exp Med* **176,** 1063.
26. Lamb J.R., Skidmore B.J., Green N., Chiller J.M & Feldman M. (1983) Induction of tolerance in influenza virus immune T lymphocyte clones with synthetic peptides of influenza haemagglutinin. *J Exp Med* **157,** 1434.
27. Lamb J. & Feldmann M. (1984) Essential requirement for major histocompatibility complex recognition in T cell tolerance induction. *Nature* **308,** 72.
28. O'Hehir R. & Lamb J. (1990) Induction of specific clonal anergy in human T lymphocytes by *Staphylococcus aureus* enterotoxins. *Proc Natl Acad Sci USA* **87,** 8884.
29. Sidhu S., Bal V., Batchelor J.R., Lombardi G. & Lechler R.I. (1992) Human T cells cannot act as autonomous antigen presenting cells but induce tolerance in antigen specific and alloreactive responder cells. *J Exp Med* **176,** 875.
30. Sansom D.M & Hall N.D (1993) B7/BB1, the ligand for CD28 is expressed on repeatedly activated human T cells *in vitro*. *Eur J Immunol* **23,** 295.
31. Pichler W.J. & Wyss-Coray T. (1994) T cells as antigen presenting cells. *Immunol Today* **15,** 312.
32. Wyss-coray T., Mauri-Hellweg D., Baumann K., Bettens F., Grunow R. & Pichler W.J. (1993) The B7 adhesion molecule is expressed on activated human T cells: functional invilvement in T–T cell interactions. *Eur J Immunol* **23,** 2175.
33. Ettinger R., Panka D.J., Wang J.K., Stanger B.Z., Ju S. & 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,** 1041.
34. Anel A., Buferne M., Boyer C., Schmitt-Verhulst A.-M. & Golstein P. (1994) T cell receptor-induced Fas-L expression in cytotoxic T lymphocyte clones is blocked by protein tyrosine kinase inhibitors and cyclosporin A. *Eur J Immunol* **24,** 2469.
35. Ju S., Panka D.J., Cui H. *et al.* (1995) Fas (CD95)/FasL interactions required for programmed cell death after T cell activation. *Nature* **373,** 444.
36. Harding F., McArthur J.G, Gross J.A., Raulet D.H & Allison J.P (1992) CD28-mediated signalling co-stimulates murine T cells and prevents the induction of anergy in T cell clones. *Nature* **356,** 607.
37. Boise L.H., Minn A.J., Noel P.J. *et al.* (1995) CD28 costimulation can promote T cell survival by enhancing expression of Bcl-X_L. *Immunity* **3,** 87.
38. Vignaux F. & Golstein P. (1994) Fas-based lymphocyte-mediated cytotoxicity against syngeneic activated lymphocytes:a regulatory pathway? *Eur J Immunol* **24,** 923.
39. Hewitt C., Lamb J., Hayball J., Hill M., Owen M. & O'Hehir R. (1992) Major histocompatibility complex independent clonal T cell anergy by direct interaction of *Staphylococcus aureus* Enterotoxin B with the TCR. *J Exp Med* **175,** 1493.
40. Dustin M.L. & Springer T.A. (1989) Tcell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* **341,** 619.
41. Klas C., Debatin K.-M., Jonker R.R. & Krammer P.H. (1993) Activation interferes with the APO-1 pathway in mature human T cells. *Int Immunol* **5,** 625.
42. Yang Y., Mercep M., Ware C.F. & Ashwell J.D. (1995) Fas and activation-induced Fas-L mediate apoptosis of T cell hybridomas: inhibition of Fas-L expression by retinoic acid and glucocorticoids. *J Exp Med* **181,** 1673.