reveals that 11 of the 17 DR1 residues in the TSST-1:DR1 interface (underlined in Table 3) are common to the SEB:DR1 interface, despite the overall difference in orientations of the two superantigens on DR1. (Thirteen of the 19 positions on TSST-1 that contact DR1 are homologous to positions on SEB that contact DR1.) Thus, it seems impossible for TSST-1 and SEB to bind simultaneously to DR1, as they would need to occupy the same space. Yet, neither TSST-1 nor SEB appears to be able to completely inhibit the binding of the other (*11*, *13*, *25*). One possible explanation for this dilemma would be the existence of a second binding site on DR1 for SEB or TSST-1, but there is no evidence for such a site.

The most striking new observation about superantigen–class II interaction seen in the TSST-1:DR1 complex is that the superantigen covers most of the peptide binding site, contacting all the polymorphic residues on the α chain α helix, residues on the bound peptide, and part of the β chain α helix, across the peptide binding site. This contrasts with our expectation because superantigen activation of T cells is reportedly much less MHC-restricted and peptide-dependent than peptide antigen–induced activation. It suggests that TSST-1 binding to DR1 may be in part peptide-dependent. Recent binding measurements between superantigens and DR molecules on different cell types suggest that different subsets of HLA-DR molecules may bind TSST-1 and SEB (*25*). Peptide-dependent binding offers a possible mechanism for superantigens to distinguish different subsets of DR1 molecules and that in turn could account for the inability of TSST-1 to inhibit completely the binding of SEB to DR1. Superantigen activation dependent on peptide (and hence also MHC allele) would allow a pathogen to direct T cell activation by its antigens or by host antigens during infections, with potential consequences for inducing specific autoreactivity.

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33. Another contact is found in the crystal between a symmetrically related *TSST-1 molecule and the TSST-1:DR1 complex. Of the six TSST-1 residues implicated in TCR recognition by mutagenesis (Y115, E132, H135, I140, H141, and Y144) (*26*), two (Y115 and Y144) are in the TSST-1:*TSST-1 contact and three (H135, I140, and H141) are in its immediate vicinity.
34. We thank D. H. Ohlendorf, J. H. Brown, and L. J. Stern for the coordinates of TSST-1 and DR1; N. Ramesh and R. S. Geha for an initial sample of purified TSST-1; P. Klimovitsky and A. Haykov for technical assistance; and M. Pietras for large-scale production of tissue culture cells. Discussions with T. S. Jardetzky, D. N. Garboczi, and A. Seth and help from D. C. Rees, P. J. Bjorkman, S. E. Ryu, M. J. Eck, R. S. Brown, R. Nolte, and C. Garnett are appreciated. J.K. acknowledges support by the Howard Hughes Medical Institute (HHMI) and NIH. R.G.U. is supported by the Irvington Institute for Medical Research. D.C.W. is an investigator of HHMI. Research supported by an NIH grant to D.C.W. Coordinates will be deposited in the Protein Data Bank and are available before their release by e-mail (kim@xtal0.harvard.edu).

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Subsets of HLA-DR1 Molecules Defined by SEB and TSST-1 Binding

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Superantigens bind to major histocompatibility complex class II molecules on antigen-presenting cells and stimulate T cells. *Staphylococcus aureus* enterotoxin B (SEB) and toxic shock syndrome toxin–1 (TSST-1) bind to the same region of human lymphocyte antigen (HLA)–DR1 but do not compete with each other, which indicates that they bind to different subsets of DR1 molecules. Here, a mutation in the peptide-binding groove disrupted the SEB and TSST-1 binding sites, which suggests that peptides can influence the interaction with bacterial toxins. In support of this, the expression of the DR1 molecule in various cell types differentially affected the binding of these toxins.

Superantigens (SAGs) are T cell mitogens produced by a variety of bacteria and viruses (*1*). The formation of a trimolecular complex between SAGs, major histocompatibility complex (MHC) class II molecules, and the T cell receptor (TCR) leads to the activation of T cells in a V_β-restricted fashion (*2*). The most studied superantigens of

Staphylococcus are the enterotoxins A (SEA), B (SEB), and TSST-1. They are globular proteins of about 30 kD (*3*) that do not require processing to be presented to T cells and that bind outside the antigen binding groove (*4*). Unlike nominal antigens, presentation of SAGs is not MHC-restricted; however, isotypic diversity and allelic polymorphism cause variations in the efficiency of binding or presentation of SAGs to T cells (*5*, *6*). Recent site-directed mutagenesis experiments revealed that residues α36 and α39 of HLA-DR are critical for the binding of TSST-1; this region is also involved in the interaction with SEB (*7*).

To characterize the binding sites of SEB and TSST-1, we expressed 12 mutant complementary DNAs (cDNAs) of HLA-DR1 α and β chains in DAP-3 cells. The mutant molecules showed little structural alterations, as evidenced by staining with a panel of 14 monoclonal antibodies (mAbs) specific for conformational epitopes or for the DR α or β chains. These mutants efficiently presented SEA to a murine $V_\beta3^+$ T cell hybridoma. DAP cells expressing the mutated MHC class II molecules were tested for the ability to bind and present recombinant SEB (rSEB) to a $V_\beta8.1^+$ mouse T cell hybridoma or TSST-1 to a $V_\beta15^+$ mouse T cell hybridoma (Fig. 1). Presentation of both toxins to the T cells is dose-dependent and varies with the level of expression of HLA-DR.

Mutation of DRα at position 13 (α-Y13V) (*8*) had opposite effects on the presentation of SEB and TSST-1 (Fig. 1, A and B), strongly reducing SEB presentation and enhancing TSST-1 stimulation. In the latter case, an optimal response was seen at all concentrations tested. This mutation had little effect on the binding of SEB, whereas a twofold increase in the binding of TSST-1 relative to SEB and SEA was observed (Fig. 1C). The α-Y13 residue is located in a hydrophobic depression previously shown to be part of the interface between SEB and DR1 (*9*). Mutation at position 18 (α-Q18P) in the same region had no effect on the presentation or binding of rSEB and TSST-1. Other substitutions on the α chain impaired the presentation and binding of both toxins. They include α-M36A and α-I63A, which are respectively located in the loop joining β strands 3 and 4 of the α1 domain and on the α helix of the DRα chain. These two residues are also part of the hydrophobic interface with SEB (*9*) and TSST-1 (*10*).

The most dramatic effect on the binding and presentation of SEB and TSST-1 was observed when α-K39 was substituted with Ser (α-K39S) or Ala (α-K39A) (Fig. 1). These mutations in the loop between strands 3 and 4 of the α1 domain on HLA-DR completely abolished the presentation of both SAGs at the highest concentration tested. Accordingly, binding of radiolabeled SEB and TSST-1 on α-K39S or α-K39A cells was not observed, whereas the binding of SEA remained unaffected (Fig. 1C). Altogether, our results show that the same residues on DR1 can contact SEB or TSST-1 and further demonstrate the overlap between the MHC class II binding sites for these SAGs.

These results are difficult to reconcile with previous observations that showed that SEB and TSST-1 do not compete for binding to HLA-DR1 and suggested that the binding sites of these two SAGs are distinct and nonoverlapping (*11*). However, we confirmed that rSEB and TSST-1 do not compete with each other for binding to MHC class II molecules and do not prevent the binding of SEA (Fig. 2A). As illustrated in Fig. 2, B and C, a 100-fold excess of unlabeled rSEB did not affect the binding of ^{125}I-labeled TSST-1, and excess TSST-1 had no effect on the binding of ^{125}I-labeled

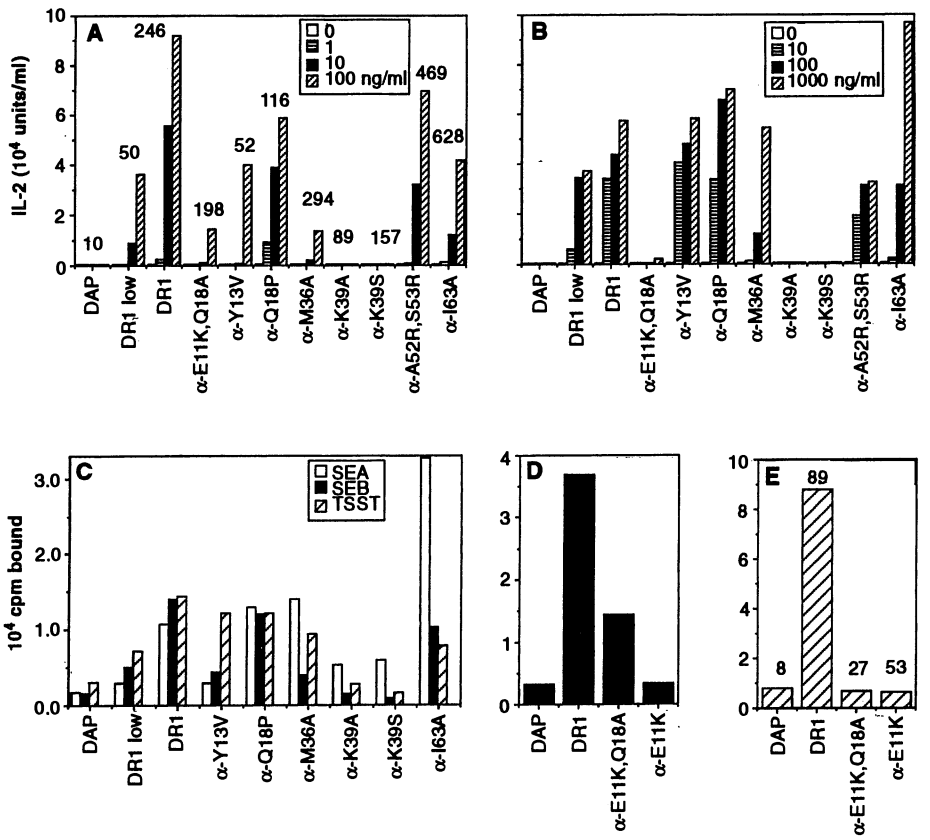

Fig. 1. Mutations on the DRα chain affect the presentation and binding of SEB and TSST-1. DAP cells expressing the wild-type DR1 molecule in high levels (DR1) or low levels (DR1 low) or the different mutants of HLA-DR1 (*32*) were used to present rSEB to the T cell hybridoma 3DT-52.5.8 ($V_\beta8.1^+$) (**A**) and TSST-1 to the T cell hybridoma KOX15.5.8 ($V_\beta15^+$) (**B**) (*33*). A different range of concentrations was used for each toxin. Each point represents the mean of triplicates. The different mutants were tested a minimum of two times with each toxin, and a representative experiment is shown. The mean fluorescence values for MHC class II expression are indicated on top of each histogram and were determined with the conformational DR mAb 50D6 (which recognizes an epitope on the β chain) (*34*). (**C**) Cells affected in their ability to present SAGs were tested in binding experiments with 200 ng of ^{125}I-labeled toxins (SEA, rSEB, and TSST-1) (*35*). The binding of radiolabeled SEB (**D**) and TSST-1 (**E**) (100 ng) was also measured on mutants of the peptide binding groove.

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rSEB to DAP DR1 cells. The presence of a second high-affinity binding site for SEB and TSST-1 is unlikely because mutagenesis of only one residue (α39) abrogates the binding and presentation of both toxins. This lack of competition between toxins that have similar binding sites is consistent with the existence of two populations of DR1 molecules, one binding to SEB and the other one binding to TSST-1. Quantitative determination of the number of toxin binding sites by Scatchard analysis with the use of the data in Fig. 2 provides evidence in support of this hypothesis. We have found 1.3×10^5 high-affinity TSST-1 receptors on DAP DR1 cells and 10^5 high-affinity SEB binding sites on the same cells. Sufficient numbers of MHC class II molecules are expressed at the surface of the DAP DR1 transfectant to account for the presence of two distinct populations of HLA-DR cells. Indeed, a minimum of 2.3×10^5 DR molecules are present at the surface of DAP DR1 cells as calculated by Scatchard analysis with the use of an ^{125}I-labeled Fab fragment from mAb L243. SEA competes efficiently for the TSST-1 and SEB binding sites (Fig. 2, B and C). It is interesting to note that the number of binding sites for SEA (2.13×10^5) is similar to the total number of sites for SEB plus TSST-1 [see above and (6)].

To better understand the molecular basis for these differences in the ability of DR molecules to interact with superantigens, we further characterized the binding sites of SEB and TSST-1 and investigated the importance of the β chain of MHC class II molecules. Mutant β-D66A,L68A was significantly affected in its ability to present TSST-1 to the $V_\beta 15^+$ hybridoma (Fig. 3B), although this mutant had little effect on SEB presentation (Fig. 3A). The lack of stimulation correlates with a reduction in TSST-1 binding as compared to SEB (Fig. 3, C and D) and is probably a result of mutation of amino acid β-D66A, which is exposed and points up toward the TCR (*12*). Our results confirm a role for the DR1 β chain in TSST-1 binding, as previously suggested (*13*). They show that the binding sites of SEB and TSST-1 are overlapping but not identical. They are also consistent with a single TSST-1 binding site composed of both α and β chain residues where TSST-1 could span the peptide-binding groove as observed in the DR1:TSST-1 crystal structure (*10*). We therefore tested the effect of mutations in the peptide-binding groove on the presentation and binding of SAGs to determine if bound peptides affect MHC:SAG interactions.

Mutations at residues 11 and 18 (α-E11K,Q18A) significantly affected the presentation of SEB and TSST-1 (Fig. 1, A and B). The same effect was observed with a DR1 molecule mutated at residue 11 alone (α-E11K) (not shown). Interestingly,

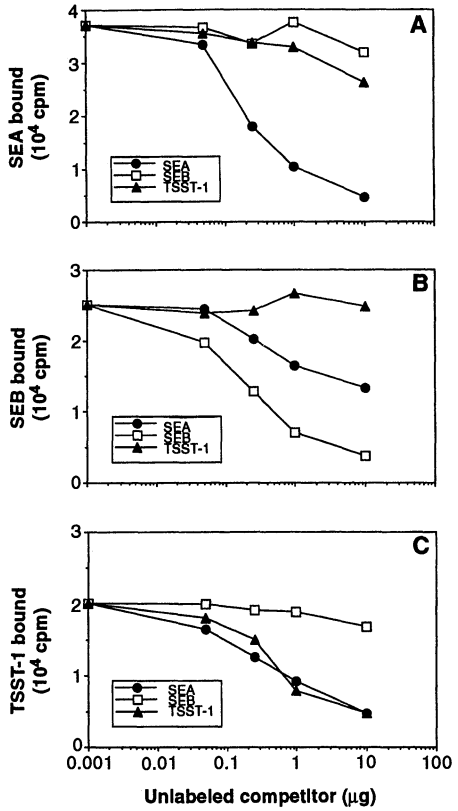

Fig. 2. SEB and TSST-1 do not compete for binding to HLA-DR. DAP DR1 cells (10^6) were incubated in 200 μl with 100 ng of iodinated SEA (**A**), rSEB (**B**), or TSST-1 (**C**) and in the presence of different amounts of unlabeled SEA, rSEB, or TSST-1. Bound counts per minute were counted, and the values were plotted against the amount of competitor toxin used. A representative experiment is shown in each panel; each point represents the mean of duplicates. These values together with those obtained in the same experiment with additional competing concentrations of toxins were used for Scatchard analysis. The ratio of unlabeled and labeled toxin specifically associated with MHC class II molecules over the unbound toxin (bound/free) was calculated and plotted against the concentration of bound toxin (bound). Linear regression analysis was performed with the Macintosh program Cricket Graph 1.3 (Cricket Software, Malvern, Pennsylvania). Correlation coefficients (R^2) of 0.89, 0.86, and 0.71 were obtained for SEA, SEB, and TSST-1, respectively. The dissociation constant for SEA was 2.3×10^{-8} M; for SEB, 1.4×10^{-8} M; and for TSST-1, 1.1×10^{-7} M.

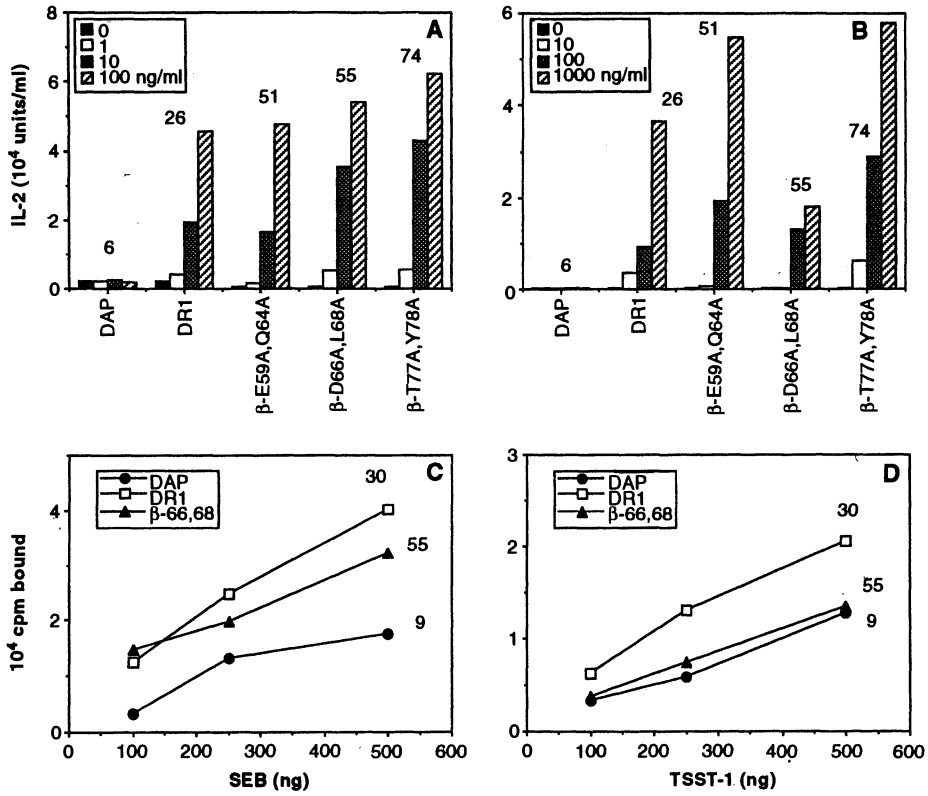

Fig. 3. TSST-1 contacts the β chain of MHC class II molecules. DAP cells expressing DR1 mutants of exposed residues on the β1 domain were tested for their capacity to present rSEB to the $V_\beta 8.1^+$ 3DT-52.5.8 (**A**) or TSST-1 to the $V_\beta 15^+$ KOX15.5.8 (**B**) T cell hybridomas. Each mutant was tested a minimum of two times; values shown represent the mean of triplicates. A representative experiment is shown. Binding experiments with radiolabeled rSEB (**C**) and TSST-1 (**D**) were performed on mutant β-66,68 (D66A, L68A), which was affected in TSST-1 presentation. Mean fluorescence values with L243 are indicated.

although both α-E11K,Q18A and α-E11K mutants abrogated the binding of TSST-1 (Fig. 1E), only the α-E11K mutant had an effect on SEB binding (Fig. 1D). The latter result could be due to a compensatory role for the Q18A mutation on SEB binding. Residue α-E11 is located under the α helix of the α chain and is part of the sixth pocket involved in the peptide binding (*14*). This residue is not in direct contact with SEB (*9*), and although it is a polymorphic residue, positively charged amino acids (as found in α-E11K) have not been reported at this position in mouse or human MHC class II molecules (*15*). Mutation of two other peptide binding residues, α52 and α53 (α-A52R,S53R) diminished the presentation (Fig. 1, A and B) and binding of both SEB and TSST-1. These residues are not involved in the SEB:DR or TSST-1:DR1 interfaces (*9*, *10*), and the effect of these mutations occurs probably through the binding of a different set of peptides to DR1 or by the induction of a novel conformation of either the bound peptide or the MHC class II–peptide complex. Such a conformational change is supported by the reduction in L243 binding for mutant α-E11K and by the loss of SEA binding for α-A52R,S53R (not shown). The effect of peptide binding on MHC conformation is not unprecedented, because the loading of MHC class I molecules with different peptides affects the reactivity of some mAbs (*16*). Moreover, the crystal structure of the H-2K^b molecule associated with the two different peptides showed some variations in both the backbone of the structure and the position of the side chains, altering the upper surface of the binding groove (*17*).

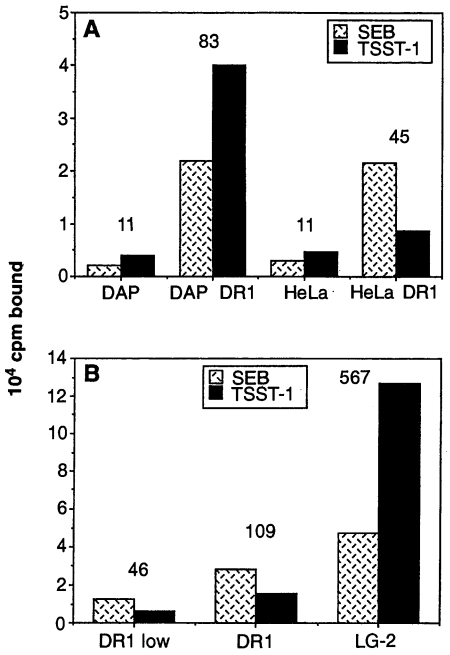

Fig. 4. The cellular background affects the ability of HLA-DR1 molecules to bind SAGs. MHC class II–negative DAP and HeLa cells were transfected with cDNAs coding for the α and β chains of DR1 and tested for their capacity to bind SAGs. Cells (10^6) were incubated with 100 ng of ^{125}I-labeled rSEB or TSST-1, and bound radioactivity was counted (**A**). DAP cells expressing different amounts of DR1 (DR1 and DR1 low) were used to compare the binding to B cells (LG-2) (**B**). Cells were stained with DR mAb 21R5, which maps to the α chain (A) or L243 (B).

Cells from various tissues generate different intrinsic peptides (*18*) that might influence the binding of SEB and TSST-1. To investigate this possibility, we studied the effect of DR1 expression in different cellular backgrounds on SEB and TSST-1 binding. Whereas DAP DR1 cells bind efficiently both SAGs, the binding of TSST-1 to HeLa DR1 cells is much less efficient than binding of SEB (Fig. 4A). In contrast, LG-2, a human B cell line that expresses large amounts of DR1, binds SEB poorly (Fig. 4B), whereas efficient binding of TSST-1 occurs on these cells. These results provide further evidence that cell-specific factors can affect the binding of ligands such as toxins or mAbs.

It is surprising that LG-2 cells, which have been used as a source of soluble MHC class II molecules for generating the DR1:SEB cocrystal, bind SEB poorly. As previously demonstrated for peptides (*19*), it is possible that soluble MHC class II molecules differ from the membrane-bound form in their capacity to interact with SEB. Interestingly, DR1:TSST-1 cocrystals could be generated only from MHC class II molecules purified by isoelectric focusing (*10*). These molecules may be loaded with specific sets of peptides. Indeed, peptides have been shown to affect the isoelectrofocusing properties of MHC molecules (*20*). It is also possible that the binding of SEB and TSST-1 to MHC class II molecules is affected by the size of the peptide, as shown for TCRs (*21*).

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32. 45.1-DRα120 (*22*), the HLA-DRα cDNA from the DRA*0101 gene, was subcloned into the Bam HI site of the Bluescript KS$^+$ vector (Promega Biotech). Mutations were introduced in the DRα cDNA by the overlap extension method (*23*), and the mutated cDNAs were sequenced. The cDNAs were inserted into the Sal I and Xba I sites of the vector RSV.5neo (*24*). The DRβ cDNA from the DRB1*0101 gene (*25*) was cloned into the RSV.3 eukaryotic expression vector. Stable transfections were carried out with the $CaPO_4$ coprecipitation method (*26*). DAP and HeLa cells were transfected with the wild-type DRβ1 and DRα cDNAs and selected in the presence of G418 (1 mg/ml). A population expressing uniform amounts of wild-type class II molecules was derived and called DR1 low. DAP cells (*27*) expressing larger amounts of class II (DR1) along with the human invariant chain p35 are described elsewhere (*28*). The mutated DRβ cDNAs were cotransfected in DAP cells with the wild-type RSV.3 DRα chain vector as described (*29*). Mutants of the α chain were transfected along with the wild-type β chain cloned in the RSV.3 or RSV.7a vectors, and cells were selected in G418 (1 mg/ml). Aseptic cell sorting was carried out on a FACStar^{PLUS} (Becton Dickinson) with DR mAbs L243 or 50D6.
33. The 3DT-52.5.8 hybridoma (*30*) is a $V_\beta1^+$ and $V_\beta8.1^+$ T cell responding to SEB and was grown in RPMI 1040 medium supplemented with 10% fetal calf serum, 10 μM β-mercaptoethanol, 2 mM L-glutamine, and gentamycin (20 μg/ml). KOX15.5.8 is a TSST-1–responsive $V_\beta15^+$ T cell hybridoma and was grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 4 mM dextrose, 2 mM L-glutamine, essential and non-essential amino acids (Gibco), 1 mM sodium pyruvate, 10 μM β-mercaptoethanol, and gentamycin (20 μg/ml). The Epstein-Barr virus (EBV)–transformed Raji cells (DR3, DRw10) (American Type Culture Collection), LG-2 cells (DR1, Dw1), and HeLa

cells were grown as above in RPMI 1040. Transfectants expressing MHC class II molecules were used as antigen-presenting cells (APCs) to stimulate T cell hybridomas in the presence of SEB or TSST-1. Briefly, 2×10^4 APCs were incubated in the presence of 6×10^4 T cells at various concentrations of toxins (0 to 1 μg/ml). Co-cultures were performed in a final volume of 200 μl in 96-well flat-bottom plates for 18 hours at 37°C in 5% CO_2. Stimulation was evaluated by the amount of interleukin-2 (IL-2) released by the T cells in the co-culture supernatants. Amounts of IL-2 were determined by the ability of the co-culture supernatant to support the proliferation of the IL-2–dependent cell line CTLL-2 and were measured with the hexosaminidase colorimetric assay (*31*).

34. Stainings were done on 5×10^5 cells for 1 hour on ice, washed twice with cold phosphate-buffered saline (PBS), and incubated for another hour on ice with fluorescein isothiocyanate–conjugated goat antibody to mouse (Caltag Laboratories, San Francisco). Flow cytometric analysis was performed on a FACScan (Becton Dickinson) with a four-decade logarithmic scale. Live cells were gated by light scatter.
35. Twenty micrograms each of SEA, TSST-1 (Toxin Technology, Sarasota, FL), and recombinant SEB were labeled for 10 min in PBS with 0.5 μg of Iodogen (Pierce, Rockford, IL) and 250 μCi of ^{125}I-Na (Amersham, Toronto) in a final volume of 60 μl. Radiolabeled products were separated from free iodine by Sephadex G-25 (Pharmacia, Sweden) exclusion chromatography in PBS containing 0.01% sodium azide and 0.1% bovine serum albumin. Specific activities varied between 7.5×10^6 and 18×10^6 cpm/μg. Adherent cells (DAP and HeLa) were trypsinized and counted. Cells (10^6) were incubated in Eppendorf tubes with 100 ng (unless otherwise specified) of radiolabeled toxins in a final volume of 200 μl of binding buffer (DMEM supplemented with 2% fetal calf serum and 0.1% NaN_3). Each point was done in duplicates, and each mutant was tested a minimum of two times with different preparations of iodinated toxins. For competition experiments, cold competitors (0.05 to 50 μg/ml) were added before the addition of labeled toxins. After 4 hours of incubation at 37°C, cells were transferred to a fresh tube over a 200-μl cushion of oil (84:16, silicone oil:mineral oil) and spun down for 1 min in an Eppendorf centrifuge. The tubes were immediately frozen at −80°C, and the tips of the tubes containing the cell-bound ^{125}I-labeled toxins were cut off and counted on a gamma counter.
36. We thank J. W. Kappler and P. Marrack for discussions and for the generous gift of recombinant SEB and the T cell hybridomas. The LG-2 cells were from L. Stern, Harvard University. We also thank J. Fraser for recombinant SEB and SEA. We are grateful to D. C. Wiley, F. Denis, and S. Gratton for discussions and critical reading of the manuscript; C. Lemire for expert secretarial assistance; C. Cantin for cell sorting; and H. McGrath, J.-P. Fortin, and H. Soudeyns for technical support. J.T. is supported by a postdoctoral fellowship from National Health Research and Development Program. N.L. is a student fellow of the Medical Research Council (MRC) and of the Université de Montréal. P.M.L. is supported by studentships from Fonds de la Recherche en Santé de Québec and MRC. R.-P.S. holds an MRC Scientist Award. This work was supported by grants to R.-P.S. from National Cancer Institute of Canada and MRC.

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Specification of C/EBP Function During *Drosophila* Development by the bZIP Basic Region

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The biologically relevant interactions of a transcription factor are those that are important for function in the organism. Here, a transgenic rescue assay was used to determine which molecular functions of *Drosophila* CCAAT/enhancer binding protein (C/EBP), a basic region–leucine zipper transcription factor, are required for it to fulfill its essential role during development. Chimeric proteins that contain the *Drosophila* C/EBP (DmC/EBP) basic region, a heterologous zipper, and a heterologous activation domain could functionally substitute for DmC/EBP. Mammalian C/EBPs were also functional in *Drosophila*. In contrast, 9 of 25 single amino acid substitutions in the basic region disrupted biological function. Thus, the conserved basic region specifies DmC/EBP activity in the organism.

Eukaryotic transcription factors influence all aspects of development by regulating gene expression. For many transcription factors, the functional interactions with cognate DNA, the transcription initiation complex, and other regulatory proteins have been studied in detail. Such analyses are usually done by transfection of tissue culture cells and in vitro assays. The ultimate goal is to determine how transcription factors function in the organism and thus which interactions are biologically relevant. Here a transgenic approach was used to directly analyze the functional requirements of the DmC/EBP transcription factor during development.

C/EBP transcription factors are characterized by their amino acid sequence similarity in the COOH-terminal DNA-binding domains (*1*–*5*). Several distinct C/EBP family members have been identified in mammals (*1*–*4*). DmC/EBP was first identified as the protein encoded by *slow border cells*, a locus required during *Drosophila* oogenesis (*5*). Subsequently, DmC/EBP was found to be an essential gene and likely to be the only C/EBP in *Drosophila* (*6*). DmC/EBP is required during late embryogenesis and may perform functions similar to those of mammalian C/EBP (*6*).

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The introduction of an ectopic copy of the gene encoding DmC/EBP (the Pw15 transgene in Fig. 1) by germline transformation was found to overcome the lethality associated with loss of chromosomal DmC/EBP and to rescue flies to adulthood. Antibody staining showed that the Pw15 transgene directed expression of DmC/EBP (or of a heterologous gene) in the normal DmC/EBP pattern in the embryo, although at a reduced level (*7*). These observations provided a sensitive functional assay for authentic DmC/EBP activity in the organism. Transgenes were manipulated in vitro and introduced into flies by germline transformation (*8*). Rescue of the lethal phenotype indicated that the protein encoded by the transgene could fulfill the essential function or functions of DmC/EBP.

To determine which domains of the DmC/EBP protein were important for function in vivo, mutant variants were generated and tested in the transgenic rescue assay. DmC/EBP consists of a COOH-terminal basic region–leucine zipper (bZIP) domain and an uncharacterized NH_2-terminal region (occupying three-quarters of the protein).

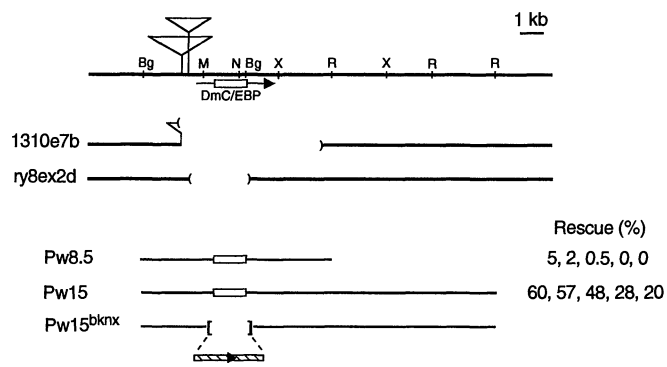

Fig. 1. Transgenic rescue assay (*8*). Top: Map of DmC/EBP genomic region. Bg, Bgl II; M, Mlu I; N, Not I; X, Xho I; R, Eco RI (only some sites are shown). P-elements associated with *slbo* alleles (*5*) are indicated as triangles, the DmC/EBP transcript is indicated by an arrow, and the open reading frame (ORF) is indicated by a white box. Represented below are DmC/EBP-null chromosomes used (1310e7b and ry8ex2d) and the fragments that were cloned into pCasPer to generate Pw8.5 and Pw15 transgenes. In Pw15^{bknx}, the DmC/EBP ORF was replaced by a linker with unique cloning sites (*23*).