

Short paper

Thomas Herrmann,
Roberto S. Accolla[○] and
H. Robson MacDonald

Ludwig Institute for Cancer
Research, Lausanne Branch,
Epalinges and Institute of
Immunological Sciences[○],
University of Verona, Policlinico di
Borga Roma, Verona

Different staphylococcal enterotoxins bind preferentially to distinct major histocompatibility complex class II isotypes

The stimulation of T cells by staphylococcal enterotoxins (SE) is strictly dependent on major histocompatibility complex (MHC) class II-bearing cells. The interaction between SE and MHC class II molecules was studied on the human B cell lymphoma Raji and its MHC class II-negative variant RJ 2.2.5. Affinity purification with SEA and SEB matrix allowed the isolation of HLA-DR-like molecules from detergent lysates of ¹²⁵I surface-labeled Raji cells, but not from RJ 2.2.5 cells. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis also revealed preferences in the binding of other SE such as SED, SEE and toxic shock syndrome toxin 1 to DR-like molecules, SEC2 to HLA-DQ-like molecules and SEC3 to DR- and DQ-like molecules. Preadsorption of the different MHC class II MHC isotypes confirmed the preferential binding of SEA to DR and of SEC2 to DQ. The implications of these findings for the understanding of SE-induced T cell activation and the potency of SE as a tool in the study of MHC class II antigens are discussed.

1 Introduction

The mitogenic potency of *Staphylococcus aureus* enterotoxins (SE) for lymphocytes was first described almost 20 years ago [1]. Recent studies have revealed some functional T cell-activating properties unique to these substances and resembling the mitogen of *Mycoplasma arthritidis*: (a) the dependence on the presence of MHC class II-bearing cells, but not on the species or the haplotype of the MHC class II-positive cells [2–4]; (b) the clonal restriction of the stimulated T cells [4, 5]. Additionally a strong correlation between the V_β elements of the TcR of the responding T cells and the SE used for stimulation has been found [6–8]. This, and the finding that neonatal treatment with SE leads to intrathymic deletion of SE-reactive T cells [6], resembles the processes involved in the generation of self tolerance [9, 10].

Working on the hypothesis that there might be a direct interaction of SE with MHC class II molecules, the binding of biotinylated SEA and SEB to the human lymphoblastoid B cell line Raji and its class II-negative variant RJ 2.2.5 was tested. Binding of SEA and SEB to HLA-DR was directly demonstrated by affinity purification of HLA-DR from detergent lysates using a SEA/SEB affinity matrix, confirming the findings recently obtained by others [11–13]. In addition SDS-PAGE of detergent-solubilized and SE affinity-purified molecules suggested certain preferences of different SE for different MHC class II isotypes (DR, DQ). In two cases this preference was directly confirmed by

removal of the relevant MHC molecules prior to isolation of the SE-binding structures.

2 Materials and methods

2.1 Cell culture

The human B cell lymphoma Raji and its MHC class II-negative variant RJ 2.2.5 [14] were used. Cells were propagated in DMEM, 7% FCS, 10 mM Hepes, 1 mM L-glutamine and 5×10^{-5} M 2-ME at 37°C, 5% CO₂.

2.2 mAb and SE

The mAb used recognize monomorphic determinants of human MHC antigens; D1-12 [15] on DR, B7/21 [16] on DP and B9.12.1 on HLA-A, B, C [17]. BT3/4 is specific for DQ1 [18]. The SE were obtained from Toxin Technology, Madison, WI.

2.3 Biotinylation of SE

The biotinylation was performed by incubating 0.3 to 1 mg/ml SE in borate buffer (0.2 M, adjusted with NaOH to pH 8.3) with a 20- to 30-fold molar excess biotin-X-NHS (Calbiochem, San Diego, CA) for 2 h at room temperature and was stopped by addition of 5% (v/v) 1 M Tris-HCl, pH 7.4. A 20–50-fold concentrated stock of biotin-X-NHS was prepared immediately before use by dissolving it in DMSO. Buffer changes and removal of free biotin-X-NHS was performed by ultrafiltration with Centricon 10 micro-concentrators (Amicon-Grace Industrial Chemicals, Lausanne, Switzerland). This allowed the use of small amounts of SE (30 µg in 100 µl reaction mixture) and recovery after the reaction in a small volume, without carrier proteins during the buffer exchange. For a buffer exchange of an initial volume of 100 µl 3–4 centrifugations (45 min, 5000 × g, 4°C) with 1–2 ml buffer were performed.

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Correspondence: H. Robson MacDonald, Ludwig Institute for Cancer Research, Ch. des Boveresses, CH-1066 Epalinges, Switzerland

Abbreviations: Biotin-X-NHS: Biotinyl-ε-aminocaproyl N-hydroxysuccinimide ester ExFT: Exfoliative toxin SE: *Staphylococcus aureus* enterotoxin TSST-1: Toxic shock syndrome toxin 1

2.4 Preparation of the affinity matrix

Approximately 3–5 µg biotinylated SE were incubated with 10 µl streptavidin-agarose (Sigma, St. Louis, MO) for 30 min in 100 µl washing buffer (0.5% NP40, 150 mM Na₂N₃, 20 mM Tris-HCl) and washed once. The immunoaffinity matrix was prepared as described [19]. The protein A-binding mAb were covalently linked to protein A-Sepharose CL4B (Pharmacia, Uppsala, Sweden) with 20 mM of the cross-linking reagent dimethyl pimelimidate dihydrochlorid (Serva, Heidelberg, FRG). For the preparation of the BT 3/4 matrix the prot A-Sepharose was precoated with rabbit anti-mouse Ig serum.

2.5 Cell surface labeling and affinity purification of cell extracts

The cell surface labeling was performed according to [20] using 1×10^7 to 1.5×10^7 cells and 18.5 mBq Na¹²⁵I (IMS 30 carrier free; Amersham Buchler, Braunschweig, FRG) for each iodogen (Bio-Rad, München, FRG)-coated vial. Extraction of membrane proteins was performed with 3×10^7 – 5×10^7 cells/ml 0.5% NP40, 20 mM Tris-HCl pH 7.4, 150 mM NaCl and a cocktail of protease inhibitors (pepstatin A, antipain, chymostatin and PMSF; Fluka, Buchs, Switzerland) for 30 min on ice. The detergent lysate was centrifuged for 15 min at $15000 \times g$, 4°C, and the SN stored at 4°C. Prior to use the extracts were preadsorbed for at least 30 min with 5% (v/v) prot A-Sepharose and centrifuged for 5 min at $15000 \times g$, 4°C. For immunoaffinity as well as for SE affinity purification 5–10 µl matrix/100 µl lysate were incubated overnight at 4°C and mixed continuously. Three subsequent incubations of 2–3 h were performed for preadsorption studies. Finally, the lysates were centrifuged, SN taken off and the matrix was washed $3 \times$ with washing buffer (0.5% NP40, 20 mM Tris-HCl, 150 mM NaCl).

2.6 SDS-PAGE

The molecules bound to the matrix were eluted and reduced by heating 10 µl matrix for 20 min at 80°C with 40 µl $2 \times$ SDS sample buffer (0.125 M Tris-HCl, pH 6.8, 2% SDS, 25% glycerol) containing 100 mM DTT. The elutants were analyzed by SDS-PAGE according to [21]. The size of the proteins was determined by analyzing low molecular mass markers (Pharmacia) in parallel. For autoradiography Kodak XAR 5 films were exposed for 2–7 days at –80°C using XAR super rapid intensifying screens (Kodak, Rochester, NY).

3 Results

3.1 Affinity purification of SE-binding molecules

Preliminary experiments revealed SEA/SEB-binding sites on Raji but not on RJ 2.2.5 using flow cytometry of cells stained with biotinylated SEA/SEB and FITC-avidin (data not shown). Subsequently, biotinylated SEA/SEB were used for a biochemical characterization of the putative SEA/SEB-binding sites. SEA/SEB-binding molecules were isolated from NP40 extracts of cell surface-iodinated

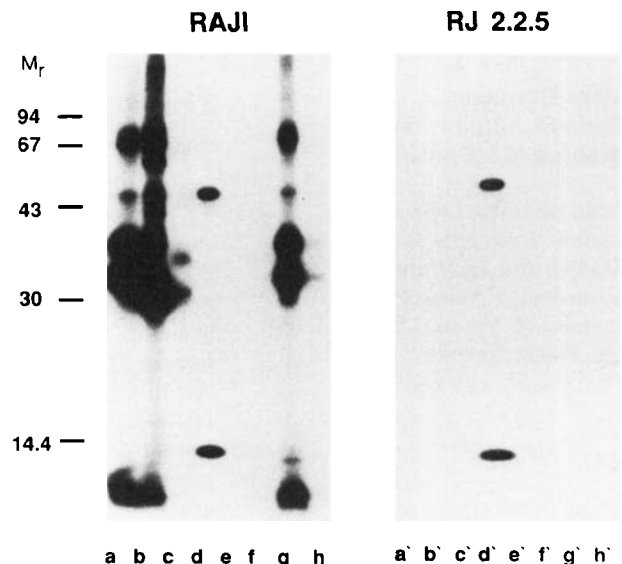


Figure 1. SDS-PAGE (12% acrylamide, reducing conditions) of SE- or immunoaffinity-purified molecules isolated from detergent extracts of ¹²⁵I surface-labeled Raji (a–h) and RJ 2.2.5 cells (a'–h'). The different matrices used are: anti-DR (a, a'), anti-DP (b, b'), anti-DQ (c, c'), anti-MHC class I (d, d'), prot A-Sepharose (e, e'), streptavidin-agarose (f, f'), SEA (g, g'), SEB (h, h').

Raji cells using an affinity matrix of biotinylated SEA/SEB coupled to streptavidin-agarose. SDS-PAGE revealed no differences between the material eluted from the SE affinity matrix and material eluted from the HLA-DR-binding matrix (Fig. 1). It had the typical pattern of DR isolated from surface-iodinated Raji cells and analyzed under reducing conditions; two strong bands of 35 kDa (α) and 31 kDa (β) and a faint band of 60–65 kDa representing the α/β heterodimer. Compared to the DR β, DP β migrates somewhat faster and DP α does not label as efficiently as DR α [22]. DQ α and β both migrate faster than DR α and β and the labeling is less intense [22]; also the precipitation is often not as efficient as for the other isotypes. The 31-kDa band of SEA-binding material also had a faint band running in parallel to the second DP β chain [22], which could not be seen in the anti-DR precipitate. The SEA matrix was much more efficient for the isolation of the DR-like molecules than the SEB matrix. No SEA/SEB binding material and no MHC class II molecules were isolated from RJ 2.2.5 cells (Fig. 1). Additional bands of 45 kDa and 12 kDa clearly coprecipitated with the MHC class II molecules, since they were not isolated from RJ 2.2.5 extracts (Fig. 1).

3.2 SE show preferences in their binding to MHC class II isotypes

Eight different biotinylated SE bound to streptavidin-agarose were used to isolate SE-binding molecules from Raji cells. One of the eight, the ExFT, did not bind any material, which might be due to the biotinylation procedure, and does not necessarily prove the incapability of ExFT to bind to MHC class II molecules. The remaining seven precipitated MHC class II-like molecules. As seen in Figs. 1 and 2 the predominant molecules binding to SEA, SEB, SED, SEE and TSST-1 resemble immunoprecipitated

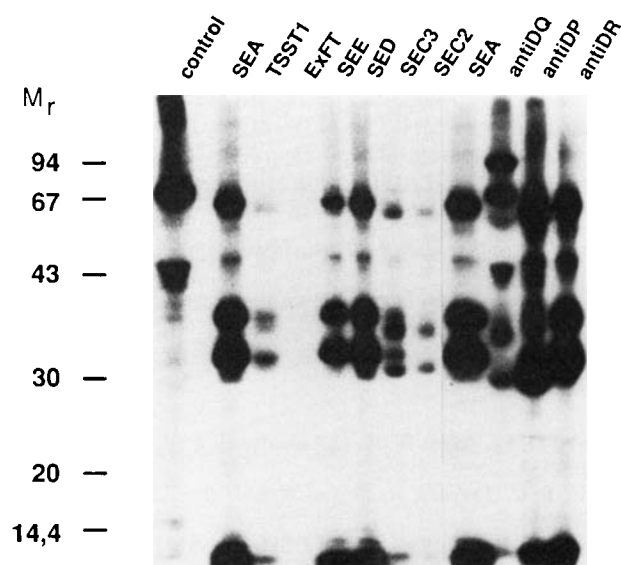


Figure 2. Isotype preferences of SE: SDS-PAGE (11.5% acrylamide) of molecules isolated from detergent extracts of ^{125}I surface-labeled Raji cells by the use of immuno or SE affinity matrix. The different matrices used are indicated above the figure. Control is a mixture of streptavidin-agarose and prot A-Sepharose precoated with mouse Ig.

HLA-DR molecules, whereas the molecules binding to SEC2 run in parallel with DQ. SEC3 bound both DR- and DQ-like molecules. SEA, SEB, SED, SEE and TSST-1 showed also faint bands running slightly faster than the strong DR-like bands.

The identity of the SE-binding material with HLA-DR, -DP or -DQ was investigated more thoroughly using SEA

and SEC2 as examples, since they seem to be specific for different MHC class II isotypes. The SE-binding molecules were identified by adsorption of the detergent extracts with DR-, DP- or DQ-specific mAb prior to affinity purification with the SE matrix (Fig. 3). Preadsorption with DR-specific mAb D1-12 almost completely removed D1-12-binding DR molecules and significantly reduced SEA-reactive material. The remaining SEA-reactive material migrated parallel to DR and the material running in parallel to the second β chain of DP was easier to detect. The disappearance of this latter material after preadsorption with DP-specific mAb suggests its identity with DP molecules. These DP molecules could be either coprecipitated with DR molecules or binding SEA with a lower affinity than DR does. The preadsorption with DQ-specific mAb seemed to have no effect on the SEA-reactive material. The isolation of the DQ-like material by the SEC2 matrix was not affected by preadsorption with either DR- or DP-specific mAb. Preadsorption using a DQ-specific mAb, however, reduced the intensity of the SEC2-binding material to a similar extent as for the material bound by the DQ mAb itself. This suggests that the SEC2-binding molecule is DQ.

4 Discussion

The interaction of SE with MHC class II molecules was studied by affinity purification of SE-binding molecules from detergent extracts of surface-iodinated Raji cells. DR-like molecules could be identified as high-affinity binding sites for SEA and SEB, confirming recent results obtained by others [11–13]. The bands of 45 and 12 kDa coprecipitated with the class II molecules could be the heavy chain of MHC class I and its light chain β_2 -microglobulin, but there is no direct proof for this assumption. A coprecipitation of MHC class I heavy chain with SEA and on one cell line with SEA as well as with DR-specific mAb has been described by Frazer [13], but in any case there is no evidence for direct SE binding to MHC class I, since neither the 45-kDa nor the 12-kDa band could be detected in precipitates of RJ 2.2.5, and since MHC class I-negative Daudi cells reconstitute T cell stimulation by SEA and SEB (Herrmann et al., unpublished data [11, 13]).

The feasibility of SE affinity chromatography using a biotinylated SE-streptavidin matrix allowed us to compare a panel of SE for MHC class II binding. In addition to SEA and SEB, SED, SEE and TSST-1 bound preferentially the human I-E homologue HLA-DR. In contrast SEC2 bound mainly the human I-A homologue HLA-DQ and SEC3 to both DR and DQ. Very recently, binding of TSST-1 to detergent-solubilized DR has also been demonstrated by others [23], but the binding of the other SE to MHC class II molecules has not (to our knowledge) been examined.

The putative association of DR with SEA and DQ with SEC2 were studied more carefully by preadsorption of the different MHC class II isotypes with immunoaffinity matrix. The almost complete preadsorption of D1-12 and SEA-binding molecules by the DR-specific mAb D1-12 demonstrated that DR is the main SEA-binding structure. The fact that the D1-12 matrix preadsorbed the SEA-binding molecules less well than the D1-12-binding mole-

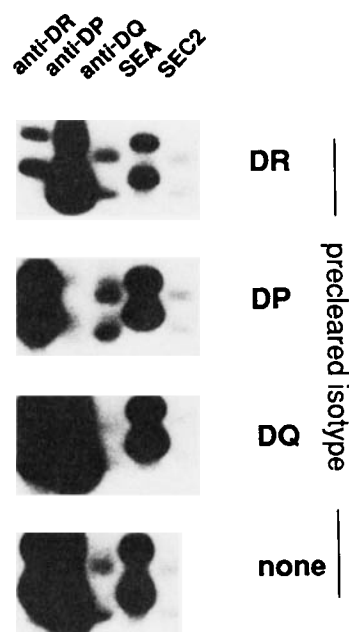


Figure 3. Determination of the SE-binding MHC class II isotypes. Detergent lysates of ^{125}I surface-labeled Raji cells were preadsorbed with immunoaffinity matrix (indicated at the right) or prot A-Sepharose. The remaining molecules were affinity purified using immuno or SE affinity matrices indicated at the top.

cules could mean that the D1-12 epitope is not expressed on all DR molecules. A certain degree of heterogeneity between the DR-(like) molecules binding to D1-12 and those binding to SEA is also suggested by preadsorption experiments with SEA; in this case the preadsorption was better for the SEA- than for the D1-12-binding material (Herrmann and MacDonald, unpublished data). Studies to test this hypothesis are underway especially to see whether SEA and D1-12 bind preferentially to different DR β chains associated with the monomorphic α chain. The fact that DP-specific mAb could preadsorb the faint lower band coprecipitated by SEA suggests that SE have preferences rather than absolute specificities for certain MHC isotypes.

Preadsorption of DQ seemed to have no effect on the SEA-binding molecules but reduced the amount of SEC2-binding molecules as it reduced the binding to the DQ-specific mAb. Therefore, SEC2 can be taken as an example of an SE having a binding preference for a MHC isotype different from DR. The fact that we and others [11] who studied detergent lysates of human EBV-transformed cells could detect no binding of SEA to DQ seems to contradict the report of Mollik and colleagues [12], who described a reconstitution of SEA mitogenicity by mouse fibroblasts transfected with human DR, DP and DQ genes, and demonstrated direct binding of SEA to DQ expressed in mouse fibroblasts. The discrepancies between the findings of the different groups might be inherent to the experimental systems used. (a) The transfected mouse fibroblasts express only one isotype of MHC class II, while the EBV-transformed cell lines express all the MHC class II subsets. Therefore, a weak binding to one subset might be difficult to detect as long as the strong binding subset is still present, even if many of the strong binding molecules are removed by preadsorption. (b) There might be differences between mouse fibroblasts and human B cells, regarding post-translational modification of MHC class II molecules, which could affect the SEA-binding properties of those molecules. (c) Allelic differences between the DQ expressed by the transfected fibroblasts and those expressed by Raji cells might also influence SE binding.

The apparent preferences of SE for binding to distinct MHC class II isotypes may have some functional consequences as well since (a) SE with preferences for different isotypes (or allotypes) could bind MHC class II-bearing cells without competition for a common binding site. (b) Differential binding of SE to MHC class II isotypes could influence the recognition of SE/MHC class II complex by T cells and (c) selective expression of MHC class II isotypes, as has been described for different tissues [25] and different T cell subsets [26], may influence the local immune response to SE *in vivo*.

Finally the high affinity of some SE for MHC class II molecules will make them powerful tools for the purification and detection of MHC class II antigens. The fact that seven of eight SE retain their MHC class II-binding properties after modification with biotin-X-NHS and the possibility to biotinylate very small amounts of SE will allow us to test the feasibility of these substances for a broad range of

applications such as the use of SE in situations where other molecules (FcR) complicate the use of antisera as well as the potential identification of as yet serologically undefined MHC class II molecules. Furthermore, comparison of binding of SE to MHC class II antigens of different iso-, allo- or xenotype might help to localize the relevant contact sites on the SE and MHC class II molecules.

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