Modulation of Antigen Processing by Bound Antibodies Can Boost or Suppress Class II Major Histocompatibility Complex Presentation of Different T Cell Determinants

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Summary

Bound antibodies can modulate antigen processing but it is not clear to what extent this affects antigen presentation. Here we show that presentation of T cell determinants in tetanus toxin can be either enhanced or suppressed as a direct consequence of antibody modulation of antigen processing in human B lymphoblastoid cells. Remarkably, a single bound antibody or its Fab fragment can simultaneously enhance the presentation of one T cell determinant by more than 10-fold while strongly suppressing the presentation of a different T cell determinant. Biochemical analysis demonstrates that both the suppressed and boosted determinants fall within an extended domain of antigen stabilized or "footprinted" by this antibody during proteolysis. These results demonstrate that bound antibodies can modulate the capture of peptides by class II major histocompatibility complex (MHC), thus manipulating the T cell response towards or away from particular determinants. Altered processing of protein-protein complexes leading to enhanced loading of class II MHC and substantially lowered thresholds for T cell activation suggests a novel mechanism that might reveal "cryptic" self determinants.

The possibility that bound antibodies might influence antigen processing and as a consequence affect its outcome at the level of presentation to T cells, has often been raised. Earlier studies showed specific effects on T cell proliferation when particular antigen-reactive antibodies were present (1–4). At the biochemical level proteolysis of protein-antibody complexes generated persistent protein fragments not seen in the absence of antibody. Such effects have been observed during in vitro digestion of antigen–antibody complexes (5, 6) and after antigen uptake into antigen-specific human B lymphocytes (7). However no mechanistic connection has been made between antibody modulation of processing at the biochemical level and possible outcomes at the level of T cell presentation.

Recently we showed that the human monoclonal antibody 11.3 which is known to alter the course of tetanus toxin processing in human B cells (7) could block the appearance of a specific T cell determinant (8). Interference with loading of this determinant (1174-1189) was seen when this antibody specificity was present either as an antigen receptor on clone 11.3 B cells or when taken up in "piggyback" fashion into other B cells or macrophages, thus demonstrating that soluble antibodies can impose dominant and systemic effects on antigen processing. We now report that this same antibody simultaneously boosts by more than 10-fold the loading of a second determinant found approximately 100 residues downstream in the tetanus toxin molecule. Further, biochemical analysis of the region of antigen stabilized by antibody during digestion shows that both the suppressed and boosted determinants fall within its "footprint."

Materials and Methods

Cell Clones, Antigens, and Antibodies. EBV-transformed tetanus toxin–specific B cell clones 11.3, 4.2, and 8.5 and autologous T cell clones KT30, KT42, KB42, and KB43 were derived and maintained as previously described (9). Tetanus toxin–specific B cell clones A46 and FC4 were also grown for antibody production. The specificity of the T cell clones has been previously described (8–10). Human monoclonal antitetanus toxin antibodies were purified from concentrated EBV culture supernatants by affinity chromatography on protein A–Sepharose, and Fab's prepared by digestion of the intact Ig with papain-agarose followed by protein A-Sepharose chromatography to remove Fc fragments. B cell clones 4.2, A46, and FC4 recognize the toxin B fragment domain (1–864) whereas clones 11.3 and 8.5 recognize conformational determinants in the C fragment domain (865–1315). Tetanus toxin (from Wellcome Biotech
or a generous gift from the Sclavo Research Center, Siena, Italy) was purified in monomeric form before use as described previously (7). Recombinant C fragment of tetanus toxin (residues 865-1315) was prepared as described (11).

Antigen Presentation Assays. Graded amounts of antigen and different monoclonal human antitetanus antibodies (1 μg/ml unless otherwise stated) were allowed to react in the wells of a flat bottom microtiter plate (Costar Corp., Cambridge, MA) for 1 h at room temperature before the addition of 2 × 10^6 T cells and 2 × 10^5 irradiated (∼5,000 rad) clone 4.2 EBV-B cells in a final volume of 200 μl RPMI 1640, 10% FCS medium. All antibodies recognize nonoverlapping epitopes and do not affect antigen uptake by clone 4.2 B cells. Alternatively, B cells were preincubated with antibody at 0°C, washed, and cultured together with varying concentrations of antibody and 2 × 10^5 T cells as above. After 48 h the cultures were pulsed with 1 μCi [3H]-thymidine (TRA.120; Amersham International, Amersham, Bucks, UK) for 16–20 h, and the radioactivity incorporated was measured after cell harvesting by liquid scintillation counting.

In Vitro Transcription/Translation and Antibody/Antigen Footprinting. Tetanus toxin C fragment was synthesized by coupled transcription/translation in the Zubay system (Promega Corp., Madison, WI) programmed with 4 μg pETtac215 (11) DNA per 40 μl reaction and supplemented with either [35S]-methionine or [3H]-leucine (Amersham). Alternatively, fragment C was purified and iodinated as described (7, 11). Translation products or iodinated C fragment were preincubated (90 min, 0°C) with 11.3, 8.5, or A46 IgG and digested with 10% (wt/wt) trypsin in 0.1 M Tris, pH 8.5. At different times the reaction was terminated by transfer to 0°C and by the addition of 2 mg/ml BSA. Either the complete reaction (A46) or the lig bound antigen digestion products (11.3 and 8.5) recovered on protein A-Sepharose were resolved by Tris-tricine SDS-PAGE (7). Those bound to the 11.3 antibody were electroblotted onto polyvinylidifluoride (PVDF) membrane (ProBlott; Applied Biosystems Inc., Foster City, CA). The labeled 17-kD, 11.3 bound fragments were detected by autoradiography, excised, and subjected to 20 cycles of automated Edman degradation in a sequencer (model 470A; Applied Biosystems, Inc.). Radioactivity in the phenylthiohydantoins derivatives obtained from each cycle was detected by gamma (35S) or scintillation (3H) counting.

Results

The 11.3 Antibody Boosts Presentation of the 1273-1284 Determinant. The tetanus toxin–specific antibody 11.3 was shown earlier to modulate the course of antigen processing both in clone 11.3 B cells and when added as a soluble piggyback antibody (7). Recently we showed that this antibody strongly suppressed the presentation of T cell determinant 1174-1189 in both B cells and macrophages (8). Since large (∼16 kD) fragments derived from the 47-kD C fragment domain (residues 865-1315) of tetanus toxin are stabilized during processing of 11.3–tetanus toxin complexes (7), it seemed possible that other T cell determinants in this region might also be affected by binding of this antibody. To gain precise control over antibody effects on presentation, we used a single tetanus toxin–specific B cell clone (4.2) as the APC and modified the substrate processed by these cells by titrating

1 Abbreviation used in this paper: PVDF, polyvinylidifluoride.
indicate that a single antibody can boost the production of one T cell determinant (1273-1284) while simultaneously suppressing a second determinant between residues 1174 and 1189. To demonstrate this directly we titrated the level of piggybacked 11.3 Ig while keeping the antigen load constant. T cell clones specific for both the above determinants as well as for the determinant 947-967 (10) were used to monitor the fate of the C fragment/11.3 Ig substrate. A stimulation index of 1.0 defined the level of T cell proliferation in the absence of added Ig. As an increasing proportion of the antigen load became complexed to 11.3 Ig, presentation of the 1273-1284 determinant was strongly stimulated while at the same time, presentation of the 1174-1189 determinant was suppressed (Fig. 2, A and C). At an Ig concentration of 0.5 μg/ml, presentation of determinant 1273-1284 was some 20-fold enhanced relative to 1174-1189, whereas presentation of determinant 947-967 was completely unaffected (Fig. 2, C). None of these determinants was affected when a different tetanus toxin–antibody complex was offered as a substrate (Fig. 2, B and D). These results provide the first demonstration that a single antibody can modulate the outcome of antigen processing both positively and negatively for different T cell determinants.

The Footprint of the 11.3 Antibody Includes both Suppressed and Boosted Determinants. We reasoned that the mechanism underlying these antibody-mediated effects on antigen presentation might be clarified by analysis of the distinct tetanus toxin fragments known to persist in the presence of 11.3 Ig (7). Tetanus toxin C fragment–11.3 antibody complexes were allowed to form and then subjected to controlled protease

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**Figure 1.** Soluble 11.3 antibody selectively enhances presentation of the 1273-1284 determinant by B cell clone 4.2. (a) Proliferative response of clone KB42 (1273-1284 specific; □) clone KT30 (947-967 specific; ▽) to tetanus toxin presented by tetanus toxin-specific EBV-B cell clone 4.2 in the absence (□) or in the presence of antitetanus Ig 11.3 (■), A46 (△), 4.9 (○), 8.5 (●) all at 1 μg/ml (7). Proliferative response (c) of T cell clone KB42 (1273-1284 specific) to tetanus toxin–specific EBV-B cell clone 4.2 in the absence (□), or in the presence of antibody 11.3 (■) or Fab 11.3 (○) at 1 μg/ml. Proliferative response (b) of T cell clone KB42 (1273-1284 specific) to tetanus toxin (□) or peptide 1273-1284 (○, ▲) presented by B cell clone 4.2 in the absence (□), or in the presence of antibody Ig 11.3 (■, ▲) at 1 μg/ml. (b) Proliferative response of DRw52a-restricted T cell clones KB42 (□) and KT42 (▲) to tetanus toxin in the absence (□) or presence (■) of 11.3 antibody.

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**Figure 2.** Simultaneous boosting and suppressive effects on different T cell determinants by a single antibody. B cell 4.2 was incubated in the presence of either 0.6 μg/ml (A and B) or 0.15 μg/ml (C and D) tetanus toxin in the presence of variable levels of either 11.3 (□ and ▲) or A46 (△ and ○) antitetanus toxin monoclonal Ig. Ig concentrations were chosen to range from 10 times above to 10 times below that of antigen. Proliferative responses of T cell clones KT30 (947-967; ○), KB43 (1174-1189; ▲), and KB42 (1274-1289; ■) were measured, and the response in the absence of added antibody set to 1.0.
digestion in vitro. As controls, a different C fragment-Ig complex (8.5 Ig) or C fragment mixed with a nonbinding antibody (A46) were digested in parallel. The C fragment was labeled either with $^{125}$I or biosynthetically with [3H]leucine or [35S]methionine in an in vitro-coupled transcription/translation reaction programmed with C fragment DNA (11). Tryptic digestion of complexes formed between 11.3 antibody and the different labeled C fragment forms yielded a distinct 17-kD Ig-bound fragment (Fig. 3 a, lanes 5–8) similar in size to those generated after processing of the same 11.3-antigen complex in APCs (7). The control digestions produced either distinct Ig-bound fragments (Fig. 3 a, lanes 9–12), again as seen in 8.5 cells (7) or low molecular weight non-Ig associated peptides (Fig. 3 a, lanes 1–4). The 17-kD 11.3 Ig-protected fragment, labeled with $^{125}$I, [3H]Leu or [35S]Met, was blotted onto PVDF membrane and subjected to 20 cycles of automated Edman degradation. Peaks of $^{125}$I and [3H]Leu were observed at cycle 13 and at cycles 2 and 6, respectively (Fig. 3 b). No [35S] label was observed in the first 20 cycles. Inspection of the amino acid sequence of tetanus toxin C frag-
Figure 4. (a) Summary of the effects of the 11.3 Ig on three determinants in tetanus toxin. The suppressive (-) and boosting (+ + +) effects, relative to presentation by B cell 4.2, are seen when the 11.3 Ig is present either in membrane (i.e., in 11.3 B cells) or soluble (piggyback) form. Its effects are therefore dominant in other APCs. (b) Hypothetical scheme to explain the fate of the three determinants. Determinant type 1 (e.g., 947-67) falls outside the region bound stably to antibody and therefore is unaffected. Determinant type 2 (e.g., 1174-89) falls within the protected domain but either fails to be released from Ig in the class II loading compartment (MIIC) or is sterically hindered by antibody from binding class II MHC or is partially degraded before release from antibody and productive binding can take place. Determinant type 3 (1273-84) also falls within the protected or footprinted domain but now its capture is enhanced either because the antibody presents the determinant in a favorable conformation for direct class II capture as implied in the figure and/or because of increased resistance to proteolysis of this region. The membrane Ig is drawn dotted since soluble piggyback 11.3 or its Fab reproduces the same effects.

Discussion

One might predict two possible outcomes for T cell determinants located within antibody-bound antigen fragments. On the one hand loading onto class II MHC molecules might be disfavored due to sequestration by Ig. On the other hand the longer lifetime of such fragments within the peptide loading compartment ought to increase the probability of successful capture by class II. Remarkably, we find both outcomes are possible for different determinants within the same antibody bound fragment. This suggests that reciprocal relationships between B and T cell epitopes may be very difficult to predict except in instances of actual physical overlap between them (3). In the context of effects on T cell epitopes the footprint made by the B cell's Ig may in fact be more relevant since it is clear that a domain of antigen extending well beyond the actual contacts made with antibody can be
boosted by soluble piggybacked 11.3 Ig or its Fab, clearly. Brane Ig to class II MHC might take place directly, i.e., in favored versus disfavored T cell determinants remains to be cise structural relationship between antibody contact sites and accompanied by efficient Ig-mediated transport to the compart-

response, in other words that the determinants which domi-
determinants observed here suggests that prevailing antibody for Ig-enhanced capture of T cell determinants.

able to take advantage of such a mechanism were proposed our finding that presentation of the 1273-84 determinant is "cryptic" inasmuch as its presentation was only detectable when processing was modified by the 11.3 antibody. Several studies have documented the existence of subdominant or cryptic T cell determinants in foreign and self proteins and have shown that T cells specific for such determinants not only exist but are capable of being activated (12, 24-26). Lehmann et al. (27) have demonstrated "spreading" of a response to such cryptic self determinants after primary induction of responses to the dominant determinants in myelin basic protein in the mouse EAE model (27). Other studies show that autoreac-
tive T cells persist in the normal repertoire because their de-
terminants are inefficiently presented (28). We suggest that enhanced loading onto class II MHC as a direct result of the modulated processing of protein–protein complexes may be a novel mechanism of revealing otherwise cryptic T cell de-
terminants to which tolerance has never been established. Such a mechanism would operate independently of increased protein capture or increased levels of expression of class II MHC, adhesion, or costimulatory molecules and might arise, for example when antibodies elicited by foreign antigens cross-react with self proteins. However, such effects need not be confined to antibody–antigen complexes but might also occur after processing of other protein–protein complexes, for example, those formed between self and viral proteins (29) or protein chaperones.

We thank Andy Knight for helpful comments on the manuscript. This work was supported by the Medical Research Council and the Wellcome Trust. Address correspondence to Dr. Colin Watts, Department of Biochemistry Medical Sciences Institute, University of Dundee, Dundee DD1 4HN, UK.

Received for publication 18 October 1994 and in revised form 31 January 1995.

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