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 Fabs 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

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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 2×10^4 T cells and 2×10^4 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 antigen at 0°C, washed, and cultured together with varying concentrations of antibody and 2×10^4 T cells as above. After 48 h the cultures were pulsed with 1 μCi [^{3}H]-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 pTETtac215 (11) DNA per 40 μl reaction and supplemented with either [^{3}H]leucine or [^{35}S]-methionine (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 Igs 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 Ig 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 polyvinyldifluoride (PVDF)[1] 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 (^{125}I) or scintillation (^{35}S and ^{3}H) 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 in antigen in the presence or absence of 11.3 IgG or alternative antitetanus monoclonal Igs. Thus these antibodies, which can potentially modulate processing, are taken up bound to antigen in piggyback fashion. Autologous T cell clones were used to analyze the outcome of processing of these different antigen substrates.

When antibody 11.3 was added as the piggyback specificity we observed a striking enhancement (>10 fold) of the presentation of a T cell determinant previously mapped between residues 1273 and 1284 (10) and restricted by the DRw52a class II MHC molecule (Fig. 1 *a*, A). Presentation to a number of independently isolated T cell clones specific for this determinant was similarly enhanced (not shown). Several controls demonstrated that the lowered threshold for presentation of this determinant was not simply due to enhanced antigen uptake or to other effects which would generally augment antigen presentation. First, several other antitetanus antibodies, including another anti-C fragment specificity (8.5), had no effect on the presentation of the 1273-1284 determinant (Fig. 1 *a*, A). Second, the presentation of another T cell determinant in the C fragment (947-967) recognized by T cell clone KT30 was unaffected by the presence of the 11.3 antibody (Fig. 1 *a*, B). Third, Fab fragments of 11.3 enhanced presentation of the 1273-1284 determinant as effectively as intact Ig (Fig. 1 *a*, C) also indicating that effects on B cell signaling function were not involved and finally, the 11.3 Ig had no effect when the T cell determinant was introduced in peptide form not requiring processing (Fig. 1 *a*, D). Thus the 11.3 Ig causes a specific modulation of native antigen processing which augments T cell epitope presentation in a highly selective manner. Although this effect was most clearly dissected using 11.3 as a piggyback Ig, it could readily be seen when the same specificity was present as a membrane Ig, i.e., 11.3 B cells presented the 1273-84 determinant at significantly lower antigen concentrations compared with other B cell clones (data not shown), confirming the existence of preferred B/T cell pairings in this system (8).

The Boosting Effect Is Specific to the 1273-1284 Determinant. Two mechanisms might explain how bound antibodies enhance presentation. First, they may enhance directly by boosting the loading of a particular determinant onto class II MHC. Alternatively, they may enhance presentation indirectly, for example by suppressing the production of determinants that compete for the same MHC class II molecule (12). To distinguish between these possibilities we examined the effect of the 11.3 Ig on the presentation of another DRw52a-restricted tetanus toxin determinant, recognized by T cell clone KT42, which lies in the B fragment of tetanus toxin (1-864, reference 10). Suppression of a competing DRw52a binding sequence would be expected to enhance all determinants binding to the same class II specificity. In fact, only the 1273-84 determinant was enhanced (Fig. 1 *b*, A) and not the determinant recognized by T cell KT42 (Fig. 1 *b*, B). Thus, processing of the antigen/11.3 Ig substrate directly augments loading of the 1273-1284 determinant.

Simultaneous Boosting and Suppression of Different T Cell Determinants. Taken together with earlier data (8), these results

[1] *Abbreviation used in this paper:* PVDF, polyvinyldifluoride.

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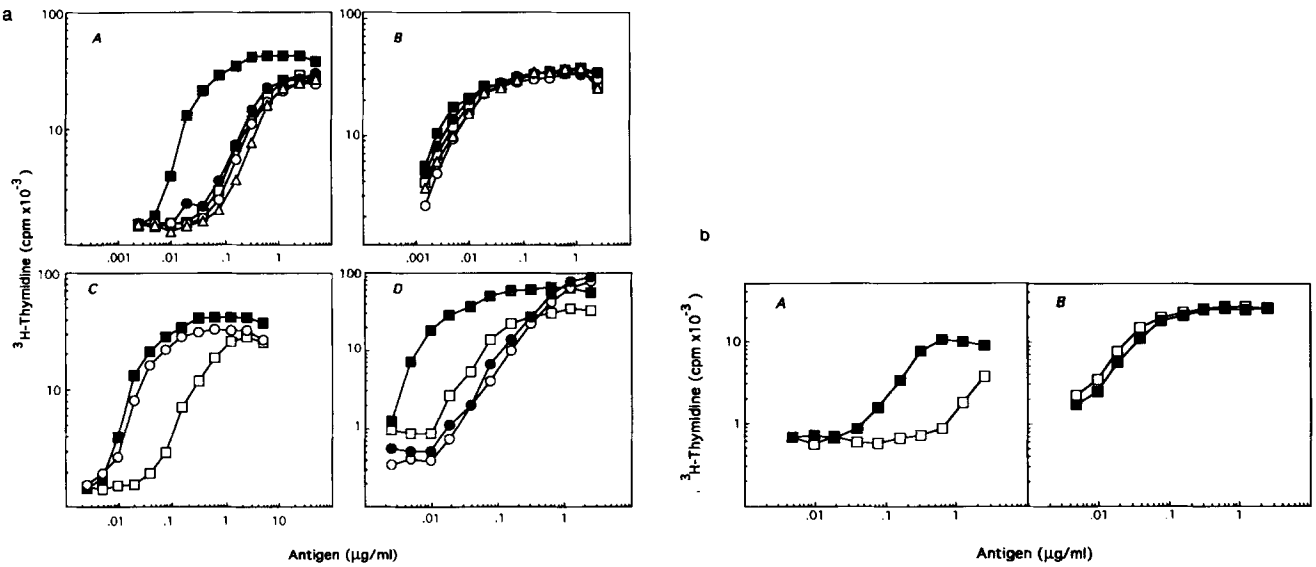

Figure 1. Soluble 11.3 antibody selectively enhances presentation of the 1273-1284 determinant by B cell clone 4.2. (*a*) Proliferative response of clone KB42 (1273-1284 specific; (A) clone KT30 (947-967 specific; (B) to tetanus toxin presented by tetanus toxin–specific EBV-B cell clone 4.2 in the absence (□) or in the presence of antitetanus Igs 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 presented by 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 (D) 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 (A) and KT42 (B) to tetanus toxin in the presence (■) or absence (□) of 11.3 antibody.

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

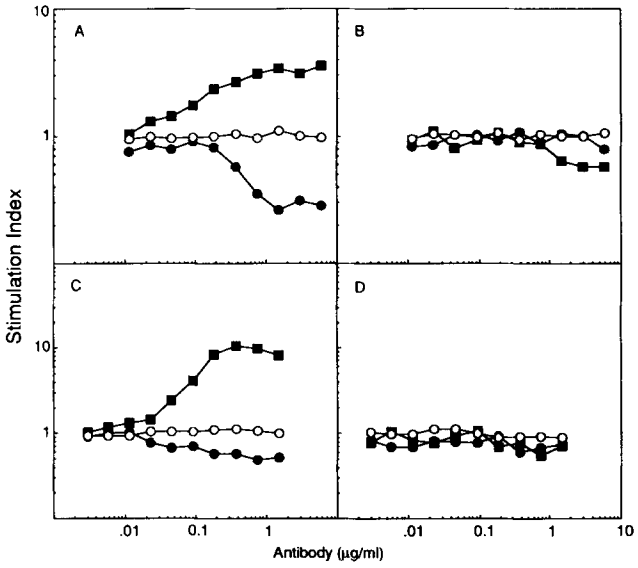

Figure 2. Simultaneous boosting and suppressive effects on different T cell determinants by a single antibody. B cell 4.2 was incubated in the presence of either 0.6 μg/ml (*A* and *B*) or 0.15 μg/ml (*C* and *D*) tetanus toxin in the presence of variable levels of either 11.3 (*A* and *C*) or A46 (*B* and *D*) 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.

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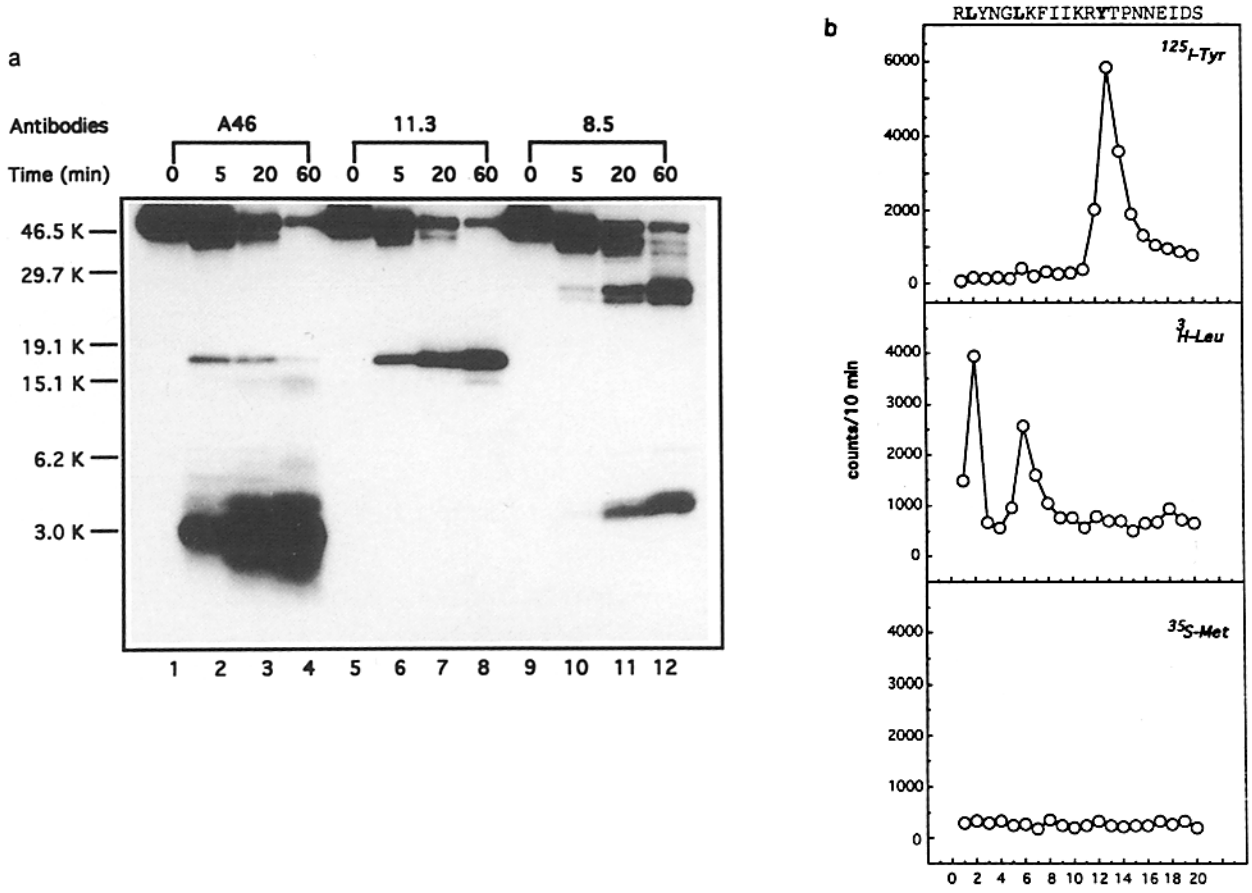

Figure 3. The "footprint" of the 11.3 antibody. (*a*) Tetanus toxin C fragment (865-1315), radiolabeled with ^{125}I or biosynthetically with [^{3}H]leucine or [^{35}S]methionine, was mixed with monoclonal antitetanus toxin antibodies 11.3, 8.5 (anti-C fragment) or A46 (anti-B fragment; 1–184) and digested with trypsin for times indicated. After termination of the digestion, aliquots of the total digestion mixture (*A46*) or the Ig-bound antigen fragments (*11.3* and *8.5*) were resolved by Tris-tricine SDS-PAGE (^{125}I digestions shown here) and transferred to PVDF membrane. (*b*) Radiosequencing of the 11.3-bound 17 kD fragment, labeled with ^{125}I, [^{3}H]Leu or [^{35}S]Met. The deduced NH_2-terminal sequence of this fragment is indicated above the top panel. (*c*) Sequence of the tetanus toxin C fragment (13, 14) showing T cell determinants 947-967, 1174-1189, and 1273-1284 (*bold*), the tryptic cleavage site defining the NH_2 terminus of the 11.3 protected fragment (*arrowed*) and the deduced region footprinted by antibody 11.3 (*boxed*). Asterisks (*) indicate the cleavage sites of fragments generated from similar digestions of 11.3/C fragment complexes with hydrolases released from frozen/thawed lysosomes purified on Percoll gradients (15).

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 [^{3}H]leucine or [^{35}S]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, [^{3}H]Leu or [^{35}S]Met, was blotted onto PVDF membrane and subjected to 20 cycles of automated Edman degradation. Peaks of ^{125}I and [^{3}H]Leu were observed at cycle 13 and at cycles 2 and 6, respectively (Fig. 3 *b*). No ^{35}S label was observed in the first 20 cycles. Inspection of the amino acid sequence of tetanus toxin C frag-

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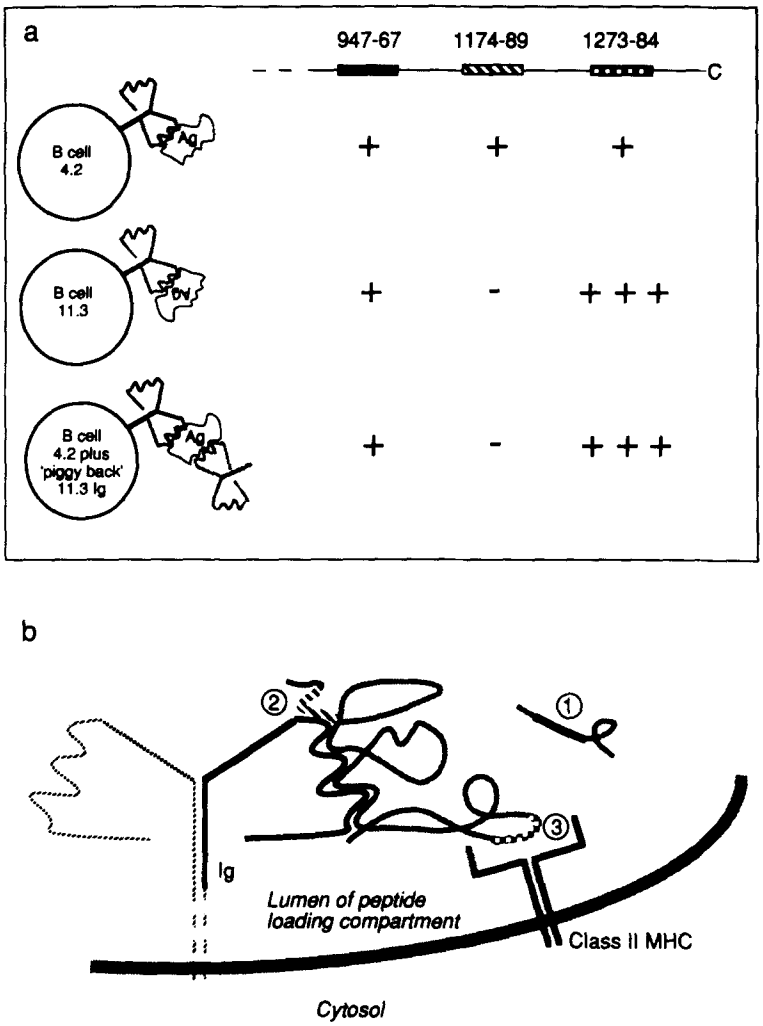

Figure 4. (*a*) Summary of the effects of the 11.3 Ig on three determinants in tetanus toxin. The suppressive (−) and boosting (+ + +) effects, relative to presentation by B cell 4.2, are seen when the 11.3 Ig is present either in membrane (i.e., in 11.3 B cells) or soluble (piggyback) form. Its effects 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.

ment (13, 14) allowed the NH_2-terminus of the 11.3 protected fragment to be unambiguously assigned to residue 1168 on the basis of this radio-sequencing data. This is deduced from the fact that of three pairs of leucines four residues apart in the C fragment only residues 1169 and 1173 are two and six residues downstream of a tryptic cleavage site and seven residues upstream of a tyrosine residue. (Note that tyrosine 1170 is apparently not labeled by the Iodogen method.) The 17-kD apparent molecular mass of the protected tryptic fragment corresponds very closely to the calculated mass of 17098.3 daltons for a fragment starting at residue 1168 and extending to the COOH-terminus of the tetanus toxin molecule. Thus the fragment of tetanus toxin footprinted by the 11.3 Ig encompasses T cell determinants whose capture can be strongly favored or disfavored by the bound antibody. Similar footprinting experiments, performed with hydrolases released from purified B cell lysosomal fractions (15), gave a somewhat smaller protected fragment of apparent molecular weight 15-kD which upon Edman degradation yielded [^{3}H]leucine and ^{125}I at cycles 14 and 18, respectively. Inspection of the C fragment sequence reveals that this fragment begins at residue 1185 which, interestingly, falls in the middle of the 1174-89 determinant suppressed by the 11.3 Ig. Since [^{3}H]leucine and ^{125}I were also observed to some extent in the two subsequent Edman cycles, fragments beginning at residues 1183 and 1184 may also exist.

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

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stabilized. The extended lifetime of this domain perhaps accompanied by efficient Ig-mediated transport to the compartment of peptide loading (16–20), might be expected to confer a global advantage to all determinants within it. However, determinants sterically constrained by antibody binding may fail to benefit. It is relevant that although the 1174-89 determinant was left intact by tryptic digestion of 11.3/C fragment complexes (Fig. 3), digestion by lysosomal hydrolases trimmed the antibody bound fragment back to residues 1184/5 destroying half of this determinant (Fig. 3 *c* and data not shown). The location of this determinant at the boundary of protease accessibility/antibody protection may explain its very poor capture by class II MHC whereas other determinants (for example, 1273-1284) may have an increased likelihood of being captured as a result of the increased lifetime of the domain as a whole. The effects of the 11.3 antibody on different T cell determinants in tetanus toxin and a hypothetical model to explain these effects is shown in Fig. 4. However, the precise structural relationship between antibody contact sites and favored versus disfavored T cell determinants remains to be established.

Based on our initial biochemical studies (7) we speculated earlier that transfer of partially processed antigen from membrane Ig to class II MHC might take place directly, i.e., in the plane of the membrane, without release into the bulk phase of the peptide loading compartment (21). Determinants able to take advantage of such a mechanism were proposed to be particularly favored. Although this remains a possibility, our finding that presentation of the 1273-84 determinant is boosted by soluble piggybacked 11.3 Ig or its Fab, clearly shows that membrane attachment of Ig is not a requirement for Ig-enhanced capture of T cell determinants.

The differential effects on the presentation of class II MHC determinants observed here suggests that prevailing antibody specificities in general may influence the focus of the T cell response, in other words that the determinants which dominate a response may do so, at least in part, through favorable antibody effects on processing at the expense of other disfavored determinants. This has been suggested before (21–23) but is dissected here for the first time at the clonal level and under conditions where antibody effects on processing are clearly distinguished from effects on uptake. It should be stressed that effects similar to those reported here are only likely to be seen when antibodies remain tightly bound at the acidic pH values found in endosomes i.e., only high affinity, class-switched antibodies are likely to be able to influence processing.

These studies demonstrate that processing of an antigen–antibody complex can substantially lower the threshold for a T cell response. As shown in Fig. 1, at low (40 ng/ml) antigen concentrations, the 1273-1284 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 autoreactive T cells persist in the normal repertoire because their determinants 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 determinants 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.

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