Brief Definitive Report

Suppressive Effect of Antibody on Processing of T Cell Epitopes

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Summary

Immunoglobulins drive efficient antigen capture by antigen presenting cells for processing and presentation on class II MHC-molecules. High affinity antibody/antigen interactions are stable at endosomal/lysosomal pH thus altering the substrate for antigen processing. We show that this can result in strong suppression of presentation of some T cell epitopes. This effect was observed when the antibody specificity was a B cell surface Ig, or formed part of an immune complex. In the latter case the presence of the suppressing antibody boosts presentation of other T cell epitopes through enhanced uptake into Fc receptor bearing cells. The influence of bound antibodies on the outcome of antigen processing may influence with T cell epitopes dominate T cell responses and may change the focus of the response with time.

The possibility that antibody binding to antigen might influence processing and consequently, presentation of T cell epitopes on class II MHC has often been debated. Pioneering studies by Berzofsky and Celada and their colleagues (1–4) indicated that antibody binding could "differentially enhance" presentation to some T cell clones either through effects on antigen uptake or on proteolytic processing. To address these questions it is essential to use a system that would allow the effects of antibody on uptake and processing to be clearly dissected and where we could analyze the effect of a single antibody specificity both as an antigen receptor on B lymphocytes and as part of an immune complex.

Earlier biochemical studies using tetanus toxin (tt)-specific human B lymphocyte clones (5) showed that high affinity antibody (Ig) binding to antigen influenced the course of antigen processing, measured by the appearance of intracellular radiolabeled antigen fragments (6). Distinct fragments were generated in B cell clones with different epitope specificity and could be modified further in the presence of additional anti-tt Fabs (6). These and other studies where antigen/antibody complexes were digested in vitro (7, 8) provided a possible biochemical basis for the idea that the spectrum of peptides generated and subsequently displayed on class II MHC molecules might be influenced by the fine specificity of the antibodies used for antigen capture (1, 2).

Here we demonstrate that antibody binding to tt can suppress the generation of a T cell epitope by a direct effect on processing, while at the same time enhance presentation of other epitopes via an effect on uptake.

Materials and Methods

Media and Reagents. The medium used throughout was RPMI 1640 supplemented with 2 mM L-glutamine, 1% nonessential amino acids, 1% pyruvate, 50 μg/ml kanamycin, 5×10^{-5} M 2-mercaptoethanol, and 10% FCS. Anti-tt antibodies (all IgG) were purified by chromatography on protein A Sepharose from concentrated culture supernatants of EBV-B cell clones. tt was a generous gift of N. Fairweather (Wellcome Biotech, Kent, UK).

B and T Cell Clones. Three tt specific EBV-B cell clones, isolated and maintained as previously described (5), were used as APC. They recognize nonoverlapping conformational epitopes which have not been precisely mapped. The B cell clone 4.2 recognizes the so-called B fragment of tt obtained by papain cleavage (1–864) and 11.3 and 8.5 recognize the C fragment (865–1315). Two additional EBV-B cell clones specific for the B fragment (A46 and 12.1) were used as a source of antibody. From the same donor from which B cell clones 4.2, 8.5, and 11.3 were isolated in 1983, a new panel of 50 T cell clones was isolated and tested for their capacity to recognize tt presented by each of the three B cell clones.

T Cell Proliferation. 2×10^4 T cells were cultured with 2×10^4 irradiated autologous EBV-B cells (6000 R) or 10^5 autologous PBMC (3000 R) in the presence of different concentrations of tt in 200 μl RPMI-10% FCS in flat-bottomed microplates. After 48 h the cultures were pulsed with 1 μCi [^{3}H]thymidine (sp act 5 Ci/mM; Amersham Corp., Arlington Heights, IL) and the radioactivity incorporated was measured after 16 h by liquid scintillation counting. In some experiments tt and anti-tt antibodies were allowed to react in the wells of a flat-bottom microtiter plate for 1 h before the addition of APC and T cells.

T Cell Epitope Mapping. 16-mers overlapping by 6 residues and spanning the whole sequence of tt C-fragment (865–1315) were

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synthesized on an Abimed AMS 422 multiple peptide synthesizer using FMOC reagents.

Results and Discussion

Identification of B Cell/T Cell Incompatibilities. To identify T cell epitopes whose presentation might be influenced by bound antibodies, we first compared the ability of three tt-specific EBV-B cell clones, each recognizing a different epitope, to present tt to a panel of tt-specific T cell clones. T cell clones KB18 and KB3 are typical of the majority of clones studied which showed no obvious B cell preference (Fig. 1, *a* and *b*). However, T cell clones KB37 and KB43 showed a strikingly different result, since they were only stimulated by B cell clone 4.2 and not by 11.3 and 8.5 (Fig. 1 *c* and *d*). Note that of the clones which showed no B cell preference, a range of sensitivity to antigen was observed, some with higher apparent affinity compared to KB43 and KB37, but also some with equal or lower affinity (see also Fig. 2).

Using overlapping peptides based on the known sequence of tt (9, 10) the epitope recognized by these clones was mapped to residues 1174–1189. Epitope 1174–1189 is found in the so-called "C fragment" domain of tt (residues 865–1315) which also includes the conformational epitopes recognized by the nonpermissive B cell clones 8.5 and 11.3. However, several other C fragment epitopes falling on both sides of the suppressed epitope are efficiently presented by these B cells; for example 947–967 (recognized by KB18) and 1273–1284 previously defined (11). Thus, in spite of the very efficient antigen uptake and presentation to most T cell clones, B cell clones 11.3 and 8.5 fail to generate the epitope 1174–1189 to a level sufficient to drive T cell proliferation. At antigen concentrations which greatly exceed that needed to saturate membrane Ig (30 μg/ml), all 3 B cell clones now presented epitope 1174–1189 equally well (data not shown) showing

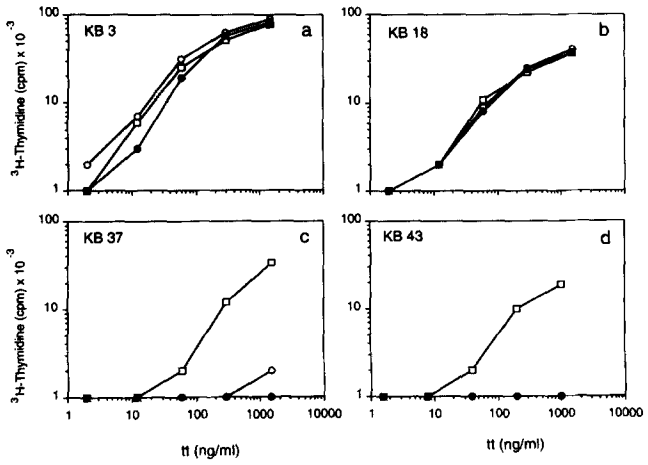

Figure 1. Differential presentation of the 1174–89 epitope by different tt-specific B cell clones. Each panel represents the proliferative response of a T cell clone to tt presented by three tt-specific EBV-B cells: 4.2 (□) 11.3 (●) and 8.5 (○). (*a*) T cell clone KB3; (*b*) T cell clone KB18, specific for (tt 947–967), (*c*) and (*d*) T cell clones KB37 and KB43 specific for tt 1174–1189.

that it is only the antigen taken up on membrane Ig which fails to generate this epitope.

These results indicate that B cell/T cell incompatibilities can be found in an autologous panel of human lymphocyte clones.

Suppressive Effect of Soluble Antibody on Antigen Presentation by Permissive B Cells. To confirm that B cell Ig specificity rather than clonal differences in antigen processing (12) accounted for the preferential B cell/T cell pairing we tested the effect of adding soluble 11.3 or 8.5 antibody to the compatible B cell/T cell combination 4.2/KB43, thus creating a situation analogous to our earlier biochemical experiments (6), where additional antibody specificities are taken up, bound to antigen, in "piggyback" fashion. As shown in Fig. 2, *a* and *b*, addition of these antibodies caused a dose dependent inhibition of presentation by clone 4.2 B cells to KB43 T cells. This suppression was very effective; such that a molar equivalent of Ig to antigen was sufficient to strongly inhibit presentation of the 1174–1189 epitope (Fig. 2). Presentation to other T cell clones was not affected even at high concentrations of these antibodies (5 μg/ml), ruling out the possibility that they were toxic or inhibited antigen uptake (Fig. 2, *c* and *d*). Thus these B cell/T cell incompatibilities can be directly attributed to antibody specificity and, importantly, the same specificity offered as soluble antibody can block the ability of an otherwise competent B cell to present antigen to a specific T cell clone.

Suppressive Effect of Antibody on Antigen Processing by FcR$^+$ APC. The suppressive effects of an antibody on processing might have a more widespread effect on the T cell response if the antibody can exert the same effect when present as part of an immune complex target to the Fc receptor bearing APC. Indeed, recent evidence indicates that priming and expansion of T cells requires professional APC such as dendritic cells or macrophages, which are more abundant than antigen-specific B cells and can deliver costimulatory signals (13–16). However, the effect of antibodies on the processing of immune complexes is complicated by the fact that they also drive antigen uptake.

Using autologous PBMC as a source of antigen presenting cells and the same T cell clones tested above, we analyzed the effect on tt presentation of adding anti-tt monoclonal antibodies, either singly or in various combinations. Presentation of tt to the T cell clone KB18 (tt 947–967) was enhanced by all antibodies tested singly and was boosted further by combining several specificities (Fig. 3 *a*) presumably due to enhanced Fc receptor-mediated antigen uptake in the presence of multiple antibodies (17). However, T cell clone KB43 showed a strikingly different response to added antibodies (Fig. 3 *b*). Some antibodies, including the "compatible" antibody 4.2, enhanced presentation, and when combined with other anti-B fragment specificities (4.2 + 12.1 + A46) presentation to KB43 was boosted still further. However, when the "incompatible" antibody 11.3 was added as a fourth specificity to the cocktail of enhancing antibodies, presentation to KB43 was virtually abolished. Only at high doses of tt, where the antibody was insufficient to complex all the antigen, could presentation to KB43 be achieved.

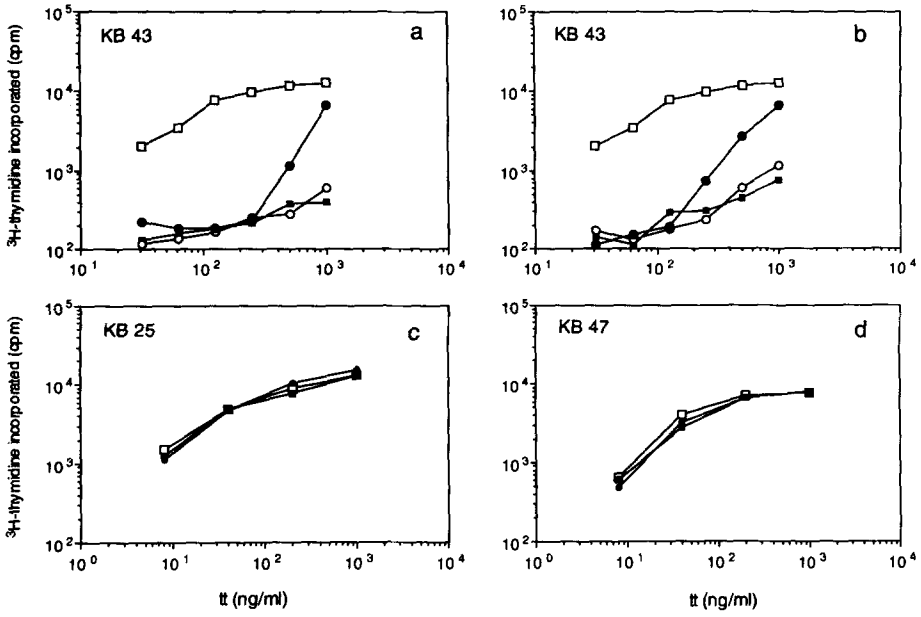

Figure 2. Soluble 11.3 and 8.5 antibodies selectively suppress presentation of the 1174–1189 epitope by B cell clone 4.2. (*a*) Proliferative response of clone KB43 (1174–1189-specific) to tt presented by B cell clone 4.2 in the absence (□) or in the presence of antibody 11.3 at 5 μg/ml (■), 1 μg/ml (○) and 0.2 μg/ml (●). (*b*) Same cultures in the presence of 8.5 antibody. (*c* and *d*) Proliferative response of clones KB25 (tt 1124–1139) and KB47 (unmapped epitope between tt 1–865) in the absence (□) or in the presence of 5 μg/ml 8.5 (●) or 11.3 (■) antibody.

Thus different T cell clones clearly illustrate the distinct effects of antibody on presentation in FcR$^+$ cells. Antibody effects on uptake are invariably positive, as previously reported by others (17, 18), and as shown here, lead to enhanced presentation of those epitopes whose processing is not affected by antibody binding. However, for other epitopes in this system (e.g., 1174–1189) the suppressive effect of antibody 11.3 on processing dominates in spite of enhanced antigen uptake, demonstrating that a single Ig-specificity can block presentation of one T cell epitope while at the same time boosting the presentation of other T cell epitopes by enhancing antigen capture by Fc receptor bearing cells.

Concluding Remarks

Using an experimental system where the same antibodies can be analyzed both as receptors on B lymphocytes and as soluble molecules in immune complexes, we have found a clear instance where antibody can suppress T cell epitope presentation due to effects on processing (Fig. 4).

In considering the possible implications of these results, it is interesting to note that the B cell producing the suppressing antibody can still receive "help" through presentation of other T cell epitopes which exist in a large antigen or protein complex (19), thus sustaining production of the suppressing antibody. In contrast, suppression of the T cell

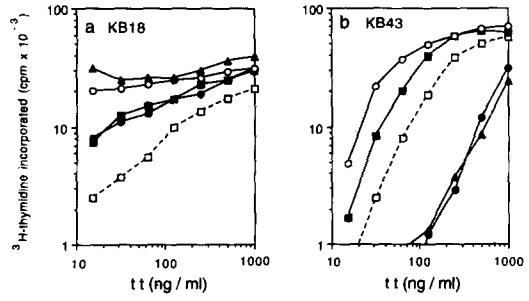

Figure 3. Different T cell epitopes are enhanced or suppressed by 11.3 antibody present in immune complexes taken up by peripheral blood mononuclear cells (PBMC). (*a*) T cell clone KB18 (947–967-specific) and (*b*) clone KB43 (1174–1189-specific) were stimulated with increasing doses of tt in the presence of irradiated (3000 R) autologous PBMC in the absence (□) or in the presence of the following antibodies: 11.3 (●), 4.2 (■), 4.2 + A46 + 12.1 (○), 4.2 + A46 + 12.1 + 11.3 (▲), each at 1 μg/ml.

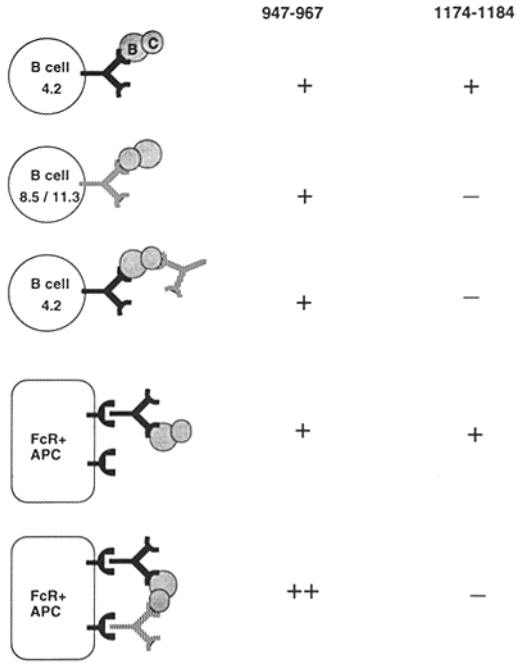

Figure 4. Differential effect of antibody on processing and presentation of two tt C-fragment T cell epitopes.

epitope will be widespread through the effect of the soluble antibody acting on other B cells and on FcR^+ cells. Thus the suppressing effect of soluble antibody imposes an asymmetric or vectorial effect on T versus B cell responses to large antigens. However, for small antigens or peptide vaccines with few T cell epitopes a suppressive effect of antibody may eventually feed back to shut down both the T and then the B cell response.

How are the 11.3 and 8.5 antibodies able to exert such a strong suppressive effect on presentation of a T cell epitope? The epitopes recognized by these antibodies are thus far only mapped to nonoverlapping conformational determinants in the tt C fragment (residues 865–1315), whereas the permissive antibody (4.2) recognizes the B fragment (residues 1–864; references 5 and 6). Since it is known that quite large fragments of antigen (>10 kD) are protected during processing by the 11.3 and 8.5 antibodies (reference 6 and P. D. Simitsek, unpublished), we suggest that these antibodies interfere with presentation by stabilizing relatively large domains, including the 1174–1189 epitope, which are not processed efficiently. Since the influence of Ig on antigen processing will eventually be overcome by further proteolytic processing, this indicates that a "window of opportunity" exists along the exogenous antigen processing pathway, within which a T cell epitope must be made available for capture by class II MHC.

Earlier studies in B cells (4) and macrophages (3) reported differential enhancement of T cell activation by different antibody specificities. In the macrophage study when mixtures of potentiating and nonpotentiating antibodies were present, potentiation of β-galactosidase presentation was dominant (3, 18). This clearly contrasts with our results which demonstrate that in some cases bound antibody can actually shut down presentation to some T cells and, moreover, does so in a dominant fashion. The tetrameric nature of β-galactosidase is likely to complicate the enhancing (via uptake) versus the potentially suppressive (via processing) effects of antibodies. The bell-shaped curves obtained in the β-galactosidase studies (3), when T cell stimulation was plotted against antibody/antigen ratio, might be due to enhanced uptake at low ratio when some subunits in the tetramer will be processed normally. At higher ratios, uptake will be enhanced further, but if all subunits are complexed presentation may be inhibited.

In mice immunized with intact influenza virus, the T and B cell repertoires to the hemagglutinin are often focused on the same regions of the antigen (20, 21). However, it is known that these B cells can receive help from many helper T cells, including those specific for other viral proteins (22). Nonetheless, it is interesting to speculate that antibody effects on processing may in some cases enhance presentation of neighboring T cell epitopes.

In general, the drive towards high affinity antibodies which do not dissociate from the antigen at endosomal pH may have a widespread influence on the outcome of antigen processing by altering the nature of the substrate for processing. We therefore suggest that prevailing antibody specificities may influence which T cell epitopes become dominant, and as different antibody specificities emerge, may change the focus of the T cell response over time.

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