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Inhibitory effects of monoclonal antibodies to a synthetic peptide of influenza haemagglutinin on the processing and presentation of viral antigens to class II-restricted T-cell clones

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SUMMARY

Monoclonal antibodies (mAb) prepared against a synthetic peptide of influenza virus haemagglutinin (HA), containing a known T-cell determinant, were used to examine the mechanism of antigen-induced activation of HA-specific class II-restricted T-cell clones. Previous studies had shown that T-cell clones, established from mice primed by infection with influenza virus, recognize variable antibody binding region of HA, including a determinant formed from residues within the sequence HA1 48–68. MAb to the synthetic peptide, p48–68, recognized purified HA and whole virus in an ELISA, and their specificity pattern for natural variant viruses was similar to that described for the T-cell clones specific for the same peptide. The anti-peptide mAb inhibited peptide or virus-induced proliferation of the peptide specific T-cell clones (but has no effect on a unrelated HA-specific clone), whereas mAb to the native HA molecule inhibited virus but not peptide-induced T-cell activation. In addition, the anti-peptide mAb showed significant inhibition of T-cell proliferation to peptide or virus pulsed antigen-presenting cell (APC). The results suggest that the anti-HA mAb affect antigen induced T-cell activation simply through blocking virus uptake by the APC, whereas the anti-peptide antibodies, which appear to recognize the same determinant on the peptide and the processed antigen, mediate their effect at the level of antigen presentation.

INTRODUCTION

The current understanding of T-cell recognition of foreign antigen suggests that T-helper (Th) cells recognize a processed form of the antigen in association with major histocompatibility complex (MHC) class II gene products on the surface of an antigen-presenting cell (APC) (Kappler & Marrack, 1976; Unanue, 1984; Schwartz, 1985). However, the precise nature of the biochemical events involved in the processing and presentation of antigen is still poorly defined (Germain, 1986; Mills, 1986). While it has been firmly established that peptide antigens do bind to purified Ia molecules (Babbitt *et al.*, 1985; Buus *et al.*, 1986a), it is not clear that the processed antigen or peptides, following their association with the MHC class II molecule on the APC, are still accessible for direct T-cell receptor interaction. Experimental attempts to address this question using antigen-specific antibodies (Ab) to demonstrate blocking of antigen induced T-cell activation have generated conflicting and somewhat paradoxical results. Monoclonal antibodies (mAb) specific for the hepatitis B surface antigen (Celis, Zurawski & Chang, 1984) or for the acetylcholine receptor (Schalke *et al.*, 1985) have been shown to potentiate activation of T cells by facilitating antigen uptake by the APC. In contrast, Ab to a variety of other antigens either did not affect T-cell response or were only inhibitory when added before antigen uptake by the APC (Ellner, Lipsky & Rosenthal, 1977; Thomas & Shevack, 1978; Glimcher *et al.*, 1983; Shimonkevitz *et al.*, 1984). However, difficulties in the interpretation of many of these findings arise from the use of polyclonal T-cell populations or antisera specific for native antigenic structures distinct from those recognized by T cells. The use of T-cell clones and mAb, specific for determinants in the same region of an antigen, should allow a more precise examination of the effects of Ab on T-cell responses.

In a previous report on the specificity of murine H-2^k class II-restricted T-cell clones for influenza virus haemagglutinin (HA), it was demonstrated that the majority of clones recognized determinant in the variable regions of the HA molecule also recognized by Ab (Mills, Skehel & Thomas, 1986a). The fine specificity of one group of clones was further characterized through their ability to recognize a synthetic peptide corres-

Abbreviations: Ab, antibody(ies); APC, antigen-presenting cell; ELISA, enzyme-linked immunoabsorbant assay; HA, haemagglutinin; HAU, haemagglutinin units; MHC, major histocompatibility complex; mAb, monoclonal antibody(ies); Th, helper T (cell).

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ponding to residue 48–68 of HA1 from the H3N2 recombinant virus, X31 (Mills *et al.*, 1988). The present study describes the specificity of mAb prepared against p48–68 and compares the effect of the anti-peptide mAb with mAb raised against the native HA molecule on antigen-induced proliferation of HA-specific T-cell clones. The results indicate that mAb, specific for a synthetic peptide with a known T-cell determinant, inhibit T-cell activation through their effect on antigen presentation, whereas the anti-HA mAb inhibit T-cell recognition by blocking virus uptake by the APC.

MATERIALS AND METHODS

Mice

CBA/Ca (H-2^k) mice were bred and housed under specific pathogen-free conditions at NIMR, Mill Hill, London and used at 2–3 months of age.

Antigens

A/X31 is a recombinant influenza virus between A/Aichi/2/68 and A/PR/8/34 with Hong Kong glycoprotein (H3N2) and PR8 internal components (Kilbourne, 1969). ENG/69, HK/71, HAN/73, TX/77, CN/84 are all H3N2 variants isolated from influenza outbreaks between 1968 and 1984, and each has several amino acid substitutions in the HA1 polypeptide resulting from antigenic drift (Wiley, Wilson & Skehel, 1981; Underwood, 1982). The laboratory mutant virus, V45, was produced by growing the parental X31 virus in eggs in the presence of the neutralizing mAb, HC45, and has a single amino acid substitution (Asp to Asn) at position 63 of HA1 (Daniels *et al.*, 1983). Viruses were grown in the allantoic fluid of embryonated hen eggs, harvested after 2 days and stored at −20°. Virus titres were determined by haemagglutination assay and expressed as haemagglutinin units (HAU)/ml. The HA glycoprotein of X31 was prepared by bromelain digestion followed by purification on a sucrose density gradient (Brand & Skehel, 1972). The synthetic peptide, p48–68, was synthesized according to the sequence of HA1 residues 48–68 of X31. This peptide was synthesized by Ralph Foulkes at NIMR, Mill Hill using a manual solid phase method developed by Merrifield (1963). Substituted peptide analogs of p49–68 were synthesized using a multiple synthesis method as previously described (Mills *et al.*, 1988). The purity of the peptides was confirmed by amino acid analysis of acid hydrolysates and by analytical HPLC.

Anti-peptide mAb

The anti-peptide mAb, 48–68C9 and 48–68B6, were derived from mice immunized with uncoupled or KLH-coupled p48–68 respectively. Conjugation of the peptide (100 μg) to KLH (200 μg) was accomplished using gluteraldehyde as a cross-linking reagent. CBA mice were immunized by i.p. injection of 100 μg of coupled or uncoupled peptide as an alum precipitate in 0·3 ml PBS containing 10^9 heat-killed *Bordetella pertusis*. The alum precipitate was prepared by adding 2 ml of 0·2 M potassium alum, dropwise with constant stirring, to a solution containing 2 ml of antigen (0·3–1·0 mg/ml) plus 1 ml of 1·0 M sodium bicarbonate. 2–3 months after the initial immunization the mice were given an i.v. boost with 20 μg of uncoupled peptide in PBS. Four days later the spleens were removed and the cells (2×10^7) fused with 3×10^7 P3-X63-Ag8.653 (JKAg8) cells using polyethyleneglycol 4000. After 2 weeks the supernatants of all wells containing a confluent growth of hybrids were screened for anti-peptide antibodies using the binding assay described below. Twelve positive wells were cloned by limiting dilution and anti-peptide specific hybridomas were grown in tissue culture to produce mAb-containing supernatants and to induce ascites in pristane-primed mice by i.p. injection of 2×10^6 cells/mouse. The ascitic fluid was collected 7–10 days later and stored at −70°C.

Anti-HA mAb

The anti-HA mAb HC45 and HC67 were derived from BALB/c mice immunized with purified X31 influenza virus as previously described (Daniels *et al.*, 1983). The specificities of the mAb were determined by sequencing the RNA of laboratory variant viruses selected by growing X31 in the presence of the mAb (Daniels *et al.*, 1983). HC67 is specific for residues Lys_{156} and Ser_{193} in antibody-binding site B. Recognition by mAb HC45 is abrogated by a single Asp to Asn substitution at position 63 in antibody-binding site E.

Enzyme-linked immunoabsorbant assay (ELISA)

For optimum binding of antigen to the wells 50 μl aliquots of virus (200 HAU/ml), HA (2 μg/ml) or peptide (10 μg/ml) in PBS was either incubated for 18 hr at 4° or dried on to the plates at 37°. After washing with PBS–Tween (PBS + 0·05% Tween 20) three times the plates were incubated for 1 hr with PBS–Tween containing 1% BSA. Anti-viral mAb were added to replicate wells, as hybridoma culture supernatants or diluted (1/100–1/1000) ascitic fluid, and incubated at room temperature for 2 hr. Plates were then washed three times with PBS–Tween and 100 μl of appropriately diluted alkaline phosphatase-conjugated goat anti-mouse IgG was added to each well and left for a further 2 hr at room temperature. Finally, after three further washes with PBS–Tween, 100 μl of phosphatase substrate (*p*-nitrophenyl phosphate, Sigma Chemical Company) were added and left for 20–60 min. The colour developed was quantitated on a automatic microelisa reader (Titrek multiskan; Flow Laboratories).

The concentration of specific anti-HA on anti-peptide antibody present in hybridoma culture supernatants or ascitic fluid was estimated by ELISA, using mAb purified by chromatography on protein A Sepharose as standards. The concentration of standard antibody was determined by spectrophotometry at 280 nm.

HA-specific T-cell clones

H-2^k class II-restricted T-cell clones were established from the spleen cells of individual CBA mice primed by infection with A/X31 influenza virus as previously described (Mills *et al.*, 1986a). Clone 18.44 recognizes a synthetic peptide, P48–68, corresponding to residues 48–68, which form part of antibody binding sites C and E, in HA1 of X31 (Wiley *et al.*, 1981; Mills *et al.*, 1986a). The recognition site of clone 13.46 has been mapped to a conformational determinant in the interface antibody-binding region (site D) (Mills *et al.*, 1986b).

T-cell proliferation assay

The T-cell clones were rested for 10–11 days after stimulation with antigen before assaying. T cells (2×10^4) were cultured with antigen (virus, 5–200 HAU/ml; HA, 1·0 μg/ml; or peptide 0·2–2·5 μg/ml) in the presence of 4×10^5 irradiated syngenic spleen

Table 1. Recognition of virus variants and synthetic peptides by mAb and a T-cell clone specific for determinants defined by residues within the sequence HA1 48–68 of the X31 influenza virus

Antigen	Sequence of residues 48–68 (48 55 60 68)	T-cell proliferation* Clone 18.44	mAb binding† 48–68B6	48–68C9	HC45
X31	T G K I C N N P H R I L D G I D C T L I D	+++	1·04	0·84	1·10
ENG/69	----------N----------	+	1·12	0·45	0·00
HK/71	----------	+++	1·21	0·68	0·57
HAN/73	----------	+++	1·65	0·83	0·41
TX/77	-------R--------D--S----------K--N----------	–	0·41	0·10	0·00
CN/84	-------R--------D--S----------K--N----------	–	0·32	0·06	0·00
V45	----------N----------	+	1·08	0·60	0·04
HA	----------	+++	0·87	0·49	0·81
P48–68	----------	+++	1·31	0·92	0·03
P49–68 (54S)	----------S----------	–	0·82	0·42	0·00
P49–68 (62K)	----------K----------	++++	0·95	0·21	0·00
P49–68 (63N)	----------N----------	+	0·68	0·52	0·01

* Data from five experiments expressed as relative response with respect to that observed with X31 (designated +++); + and ++++ indicate shifts in the dose response curve to 100-fold higher and 10-fold lower antigen concentrations respectively.

† Representative data from a typical ELISA given as net OD. readings at 450 nm after subtraction of the blank reading.

cells as APC in 225 μl volumes in flat-bottomed 96-well microtitre plates. Proliferation was measured by the uptake of [^{3}H]thymidine added 4–6 hr before the end of a 48 hr culture period. Results are expressed as mean c.p.m. for triplicate cultures from four to five experiments (SE < 20%).

Effect on mAb on T-cell proliferation

The effect of anti-p48–68 and anti-HA antibody on the activation of HA-specific T-cell clones was tested by adding 25 μl of the antibody preparation to the 225 μl cultures as for the standard proliferation assay. Unless otherwise indicated the mAb were added to cultures at a final concentration of 10% tissue culture supernatant or 0·2% ascites (equivalent to 5–20 μg/ml of specific antibody). In order to test the effect of antibody on antigen uptake, processing or presentation, the mAb was added in different combinations with antigen, T cells and APC according to the following protocols:

1. With antigen 90 min prior to the addition of T cells and APC.
2. At the same time as antigen, T cells and APC.
3. 90 min after the addition of antigen, T cells and APC.
4. With antigen-pulsed APC 90 min prior to the addition of T cells.
5. 90 min after the addition of T cells to antigen-pulsed APC.

For pulsing, APC (4×10^6/ml) were incubated with peptide (1 μg/ml) or X31 virus (200 HAU/ml) for 5 hr at 37°, washed twice, resuspended in fresh medium and cultured at a final concentration of 2×10^6/ml. Results were expressed as a percentage of the proliferation response of the T cells in the absence of mAb.

RESULTS

Specificity of mAb and T-cell clones for residues within the sequence 48–68 of influenza HA1

Murine (H-2^k) class-II restricted T-cell clones specific for the HA surface glycoprotein of the H3N2 recombinant influenza virus X31 have previously been shown to recognize variable antibody-binding regions of the HA molecule (Mills *et al.*, 1986a). Clone 18.44 is one of a group of six distinct clones which recognizes a synthetic peptide corresponding to residues 48–68 of HA1. The specificity of this T-cell clone for variant viruses and substituted synthetic peptide analogs has been described in detail elsewhere (Mills *et al.*, 1988) and is summarized in Table 1. MAb, 48–68B6 and 48–68C9, raised against the synthetic peptide (p48–68) recognized by the T-cell clones, bind to the whole virus and to the purified HA molecule when tested in an ELISA (Table 1). The specificity pattern of the mAb for natural variant viruses followed a similar pattern to that observed with the T-cell clone, 18.44. The anti-peptide mAb showed markedly reduced recognition of variants TX/77 and CN/84. Binding of mAb 48–68C9 appears to be affected by residue substitutions at position 62 and/or 63. Recognition by mAb HC45, raised against the native virus, has previously been shown to be abrogated by an Asp to Asn substitution at position 63 (Daniels *et al.*, 1983). This Ab failed to bind to p48–68 in an ELISA and may therefore recognize a conformational determinant in the region of residue 63.

Inhibition of virus or peptide induced T-cell proliferation by antigen-specific mAb

The anti-peptide mAb, 48–68B6 and 48–68C9, inhibited peptide

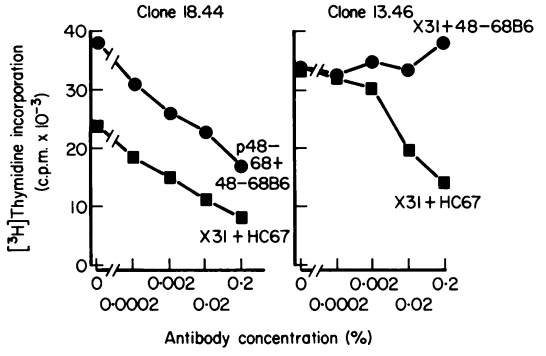

Figure 1. Inhibition of antigen-induced T-cell proliferation by anti-peptide and anti-HA mAb. T-cell clones 18.44 and 13.46 were stimulated with APC and either p48–68 (0·2 μg/ml) or X31 influenza virus (50 HAU/ml) in the presence of a range of concentrations of the anti-peptide mAb 48–68B6 or the anti-HA mAb HC67, added as diluted ascites at the initiation of culture. Proliferation was measured by [^{3}H]thymidine incorporation after 48 hr of culture. Results also mean (SE. <20%) c.p.m. for triplicate culture from a typical experiment.

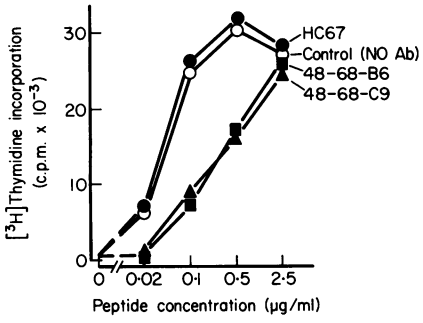

Figure 2. Effect of antigen concentration on the inhibitory effect of mAb on the T-cell proliferation response. T-cell clone 18.44 was stimulated with APC and a range of concentrations of p48–68 in the presence of the anti-peptide mAb, 48–68 8–68B6 or 48–68C9 or the anti-HA mAb, HC67. Proliferation was measured and results expressed as described in the legend to Fig. 1.

or virus-induced proliferation of the T-cell clone, 18.44, but had no effect on the response of clone 13.46, specific for an unrelated region of the HA molecule (Fig. 1). The anti-HA mAb, HC67, had no effect on the response of T-cell clone 18.44 to p48–68 (Fig. 2), but showed potent, dose-dependent inhibition of the virus-induced response of clones 18.44 and 13.46 (Fig. 1). A 50% reduction of the T-cell proliferative response to virus (50 HAU/ml) was achieved with 0·5–2 μg/ml of specific anti-HA mAb, compared with 10 μg/ml for an anti-Ia^k mAb (data not shown). An equivalent inhibition of the T-cell response to peptide (0·2 μg/ml) required 10–40 μg/ml of specific anti-peptide mAb. The antibody inhibition was overcome by increasing the concentration of stimulating antigen in the culture (Fig. 2). At a suboptimal concentration of p48–68 (0·02 μg/ml) the T-cell response, in the presence of anti-peptide mAb (20 μg/ml) was reduced to background levels, whereas at supraoptimal concentrations of peptide (≥2·5 μg/ml) the mAb had no effect.

Effect of mAb on antigen uptake and processing

The mechanism of anti-peptide and anti-HA mAb inhibition of T-cell proliferation was examined by testing the effect of addition of Ab to the antigen either: 90 min before, at the same time, or 90 min after the addition of the APC and T cells to the culture. The results in Fig. 3, expressed as a percentage of the proliferation responses in the absence of added mAb, show that the anti-p48–68 mAb inhibit the response of the T-cell clone, 18.44, to p48–68 by 60–80% and to X31 by 30–70%. The inhibition was not significantly greater when the mAb was pre-incubated for 90 min with the antigen. The anti-HA mAb, raised against the whole virus, showed a different and more complex pattern of inhibition. MAb HC45, specific for an epitope centred on residue Asp 63 in the native HA molecule, and HC67, specific for residues Lys_{156} and Ser_{193}, had no effect on p48–68-induced proliferation of clone 18.44. However, the addition of these mAb to the culture with X31 virus, either 90 min prior to or at the same time as the addition of the T cells and APC, resulted in potent (75–95%) inhibition of the T-cell response. This inhibition was either overcome (HC67) or decreased (HC45) with delayed (90 min) addition of antibody to the cultures.

Effect of mAb on antigen presentation

The effect of anti-peptide and anti-HA mAb on the presentation of processed virus or p48–68 was tested using APC pre-incubated for 5 hr with whole virus or peptide. mAb were added to pulsed and washed APC either 90 min before or 90 min after the addition of the T cells. The anti-peptide mAb inhibited the proliferation of clone 18.44 to p48–68 or X31-pulsed APC by 20–45%, but only when added before the addition of T cells to the cultures (Fig. 4). In contrast the anti-HA mAb had no affect on the T-cell response to virus or peptide-pulsed APC.

DISCUSSION

The significant findings of this study are that mAb produced against a synthetic peptide, corresponding to a region of influenza virus haemagglutinin containing a known T-cell determinant, inhibit virus- or peptide-induced proliferation of a T-cell clone specific for that determinant, whether added before or after antigen uptake and processing by the APC. In contrast anti-HA mAb raised against the native virus only inhibited virus-induced proliferation, and then only when added before antigen processing. The results suggest that the anti-HA mAb may inhibit virus uptake by the APC, whereas the anti-peptide mAb, since they still inhibit T-cell activation after allowing a period of 5 hr for antigen processing by the APC, may interfere with antigen–Ia interaction or T-cell receptor interaction with the antigen–Ia complex.

Previous attempts to use antigen-specific Ab to demonstrate inhibition of antigen-induced activation of T cells have produced largely inconsistent results. Studies with Ab to a variety of foreign antigens and synthetic haptens have reported enhancement, inhibition or no effect on T-cell proliferation or interleukin production (Ellner *et al.*, 1977; Thomas & Shevach, 1978; Glimcher *et al.*, 1983; Celis *et al.*, 1984; Shimonkevitz *et al.*, 1984; Schalke *et al.*, 1985). However, interpretation of many of the findings are complicated by the use of polyclonal Ab or heterogeneous T-cell populations. In addition, the majority of workers have only reported an inhibitory effect when the Ab was

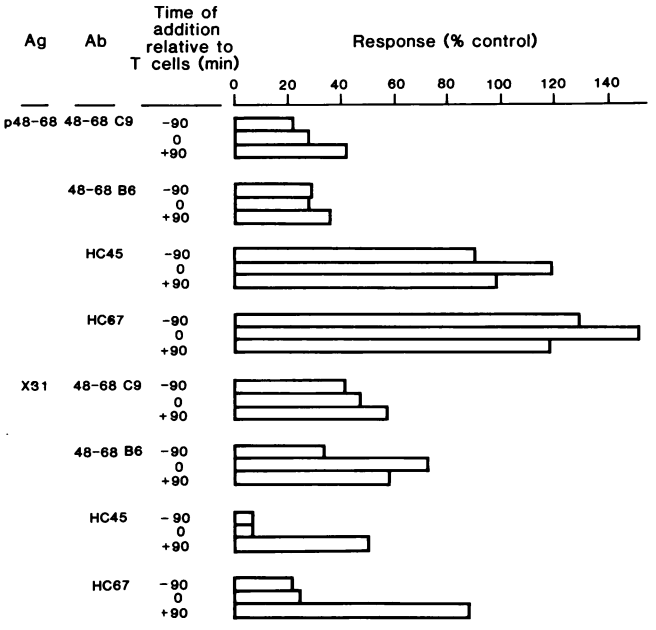

Figure 3. Effect of anti-peptide and anti-HA mAb on antigen uptake and processing. T-cell clone 18.44 was stimulated with APC and p48–68 (0·2 μg/ml) or X31 virus (50 HAU/ml) in the presence of the anti-peptide mAb 48–68B6 or 48–68C9 or the anti-HA mab HC45 or HC67. MAb preparations (25 μl) were added either 90 min before (−90) at the same time (0) or 90 min after (+90) the addition of T-cells and APC in 200 μl volumes to the culture wells containing 25 μl of the antigen preparation. Proliferation responses were measured by [^{3}H]thymidine incoporation after 2 days of culture and results are expressed as a percentage of responses in the absence of added antibody.

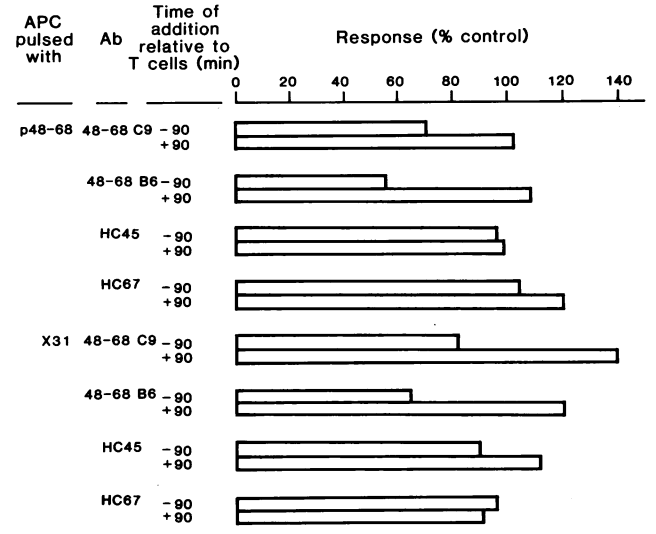

Figure 4. Effect of anti-peptide and anti-HA mAb on antigen presentation to T-cell clone 18.44. T-cells were stimulated with APC which had been pre-incubated for 5 hr with p48–68 (1·0 μg/ml) or X31 virus (200 HAU/ml). Anti-peptide mAbs 48–68C9 or 48–68B6 or anti-HA mAbs HC45 or HC67 were added to cultures with pulsed APC either 90 min before (−90) or 90 after (+90) the addition of T cells. Proliferative responses were measured and results assessed as described in the legend to Fig. 3.

added to the antigen before processing by the APC. This observation can be explained by the fact that Ab were mostly raised against native antigen and consequently may not cross-react with the linear determinant on processed antigen recognized by the majority of Th cells (Shimonkevitz *et al.*, 1984; Unanue, 1984). Two groups have addressed this problem using polyclonal antisera to small peptides also recognized by T-cell clones. Antisera to p323–339 of ovalbumin inhibited the proliferative response of T cells to limiting concentrations of peptide, but had little effect on T-cell activation by peptide or ovalbumin-pulsed APC (Shimonkevitz *et al.*, 1984). In another study, antisera to a synthetic peptide of influenza HA inhibited the T-cell response to peptide but not to the native virus (Lamb *et al.*, 1984).

In the present investigation, the availability of mAb against a synthetic peptide (p48–68), previously shown to define a class II-restricted T-cell determinant on influenza virus HA (Mills *et al.*, 1986b), along with mAb to the native HA molecule, has provided a unique system to study the effects of antigen-specific Ab on the events involved in antigen-induced T-cell activation. Like the p48–68 specific T-cell clone, 18.44, the anti-HA mAb, HC45, and the anti-p48–68 mAb were capable of discriminating between natural variant virus with amino acid substitutions at critical positions. The cross-reactivity of the anti-peptide mAb between peptide, purified HA and whole virus, as detected using an ELISA, may suggest that these Ab see the same structure on the peptide and native HA molecule. However, since they did not inhibit haemagglutination (data not shown), this conclusion remains tentative. In contrast, the anti-HA mAb, HC45 and HC67, did inhibit haemagglutination (Daniels *et al.*, 1983) and HC45, although directed at an epitope centre on a residue (ASP 63) within the sequence of p48–68, did not bind to the synthetic peptide. Consistent with the latter observations was the failure of mAb HC45 to affect peptide-induced proliferation of T-cell clone 18.44. In addition, neither anti-HA mAb affected the T-cell response to virus-pulsed APC, thus suggesting that clone 18.44 sees a processed form of the antigen which is not recognized by, or accessible to Ab to the native antigen. Conversely, the potent inhibition of virus-induced T-cell proliferation by HC45 and HC67, when added to assays before or at the same time as the APC and T cells, suggests that mAb to the native HA molecule inhibit T-cell activation by blocking virus uptake by the APC. This conclusion is consistent with the rapid and efficient binding of the influenza virus, via the HA glycoprotein, to specific sialic acid receptors present on the membrane of the majority of cells, and is supported by a recent report which demonstrated inhibition of binding of radiolabelled PR8 influenza virus to a B-cell lymphoma cell line by anti-HA mAb (Eisenlohr, Gerhard & Hackett, 1987).

The lack of inhibitory effect of antigen-specific Ab on T-cell responses, observed by other workers, may be explained by the non-specific mechanism of APC uptake of globular protein antigens or haptens, which may involve pinocytosis or phagocytosis, rather than binding to specific receptors as in the case of influenza HA. Indeed, enhancement of antigen presentation, demonstrated in a number of studies, has been explained on the basis of improved antigen uptake through the formation of immune complexes (Celis *et al.*, 1984; Schalke *et al.*, 1985). However, enhancement of T-cell response to virus or to purified bromelain-cleaved HA (Mills, unpublished results) was not observed with the anti-HA mAb. Previous failures to demonstrate inhibition of T-cell activation by Ab to whole antigens, may also be related to the fact that the Ab were directed against different regions of the molecule from those recognized by T cells. In the present study, weak inhibition of T-cell responses were still observed with Ab to the T-cell site (HC45), but not with Ab to an unrelated region of HA (HC67), when added to APC and T cells pre-incubated with antigen for 90 min. This may suggest that mAb HC45 also has an inhibitory effect on antigen processing.

The significant inhibitory effect of the anti p48–68 mAb on the response of the p48–68 specific T-cell clone to APC pre-pulsed for 5 hr with peptide or virus, followed by washing, suggests that these mAb can bind to the processed antigen, and thereby inhibit some biochemical event at a later stage of antigen presentation. Although processing of an antigen by APC can occur within 30–60 min at 37° (Unanue, 1984; Eisenlohr *et al.*, 1987), the association of peptide antigens with Ia molecules occurs relatively slowly (Buus *et al.*, 1986b). Therefore, it is possible that the anti-peptide mAb may block antigen interaction with the MHC class II molecule. However, since Ab inhibition was not observed following pre-incubation of the T cells for 90 min with the antigen-pulsed APC, it is more likely that the anti-peptide mAb block antigen interaction with the T cells. If this is indeed the case, then the processed antigen or peptide must be still accessible for Ab binding and consequently T-cell receptor interaction following its association with the class II molecule.

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