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Differential Activation of T Cells by T-Cell Receptor Ligand Analogs

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Abstract : Although $CD4^+$ T cell responses to protein-derived antigen have well been understood, the epitopes recognized by hapten-specific $CD4^+$ T cells have not been fully defined. In this study, we characterized the response of a T cell hybridoma (5D10.1B8) which is specific for a hapten, N-hydroxysuccinimidyl-4-azidobenzoate (HSAB) restricted by MHC class II I-A^d. Using three different antigen presenting cells (APCs) expressing I-A^d, the role of class II MHC proteins in haptenic antigen presentation and subsequent activation of 5D10.1B8 has been examined. Activation of 5D10.1B8 T cells by HSAB analogs was also performed. Our results show that each APC activated T cells differentially and that interleukin-2 (IL-2) augmented antigen-presenting ability of all the APCs, suggesting that increased expression of class II MHC protein by IL-2 played an important role in HSAB presentation and T cell activation. Finally, early T cell receptor-dependent signals induced by HSAB or its analogs were examined by phosphotyrosine immunoblot analysis, and showed that tyrosine phosphorylation level of a 18-20 kD protein increased upon stimulation.
Key words : hapten, T cell hybridoma, T cell receptor antagonism

Activation of $CD4^+$ T lymphocytes is a critical step in both humoral and cell-mediated immune response. $CD4^+$ T cell activation involves binding of multiple receptors on the T cell surface such as the α β T cell receptor (TCR) and a coreceptor CD4 to complexes of antigenic peptides and class II major histocompatibility complex (MHC) proteins expressed on antigen presenting cell (APC). The α β TCR binds to a polymorphic region of the peptide-MHC complex surrounding the antigenic peptide, while CD4 binds to a nonpolymorphic region of MHC (Mueller *et al.*, 1989). This activation leads to proliferation, cytokine production or cytolysis (Crabtree, 1989).

T lymphocytes, due to their selective interaction with MHC-bound peptides, are considered to be protein or peptide specific. However, MHC-restricted TCR may interact with a variety of haptens such as reactive chemicals or drugs (Pohlit *et al.*, 1979; Shearer, 1974; Weltzein *et al.*, 1996), and these reactivities are known to be responsible for hapten-induced allergies in humans and animals (Weltzein *et al.*, 1996). Therefore, the elucidation of an structural characteristics of epitope recognized by hapten-specific TCR is important for the understanding of chemical- or drug-induced allergies. However, the precise epitopes that are recognized by hapten-specific T cells are not well determined.

The recognition of hapten determinants by the immune system is believed to occur by a direct coupling of hapten to functional groups of autologous proteins such as $-NH_2$, -OH, and -SH (Eisen *et al.*, 1952). Thus, hapten like trichloronitrobenzene (TCNB) is believed to interact with the ε-NH_2 group of lysines by nucleophilic substitution, forming "hapten-protein" or "hapten-peptide" complexes (Little and Eisen, 1966). For trinitrophenyl (TNP)-specific $CD4^+$ and $CD8^+$ T cells, the antigenic determinants have been shown to be formed by TNP-modified, MHC-associated peptides (Kohler *et al.*, 1997), and the position of TNP on peptide sequence was critical for optimal recognition by TCR (Cavani *et al.*, 1995).

While the recognition of peptide antigen by TCR exhibits exclusive specificity, many studies have shown a flexibility in this recognition (Evavold *et al.*, 1991; Sette *et al.*, 1994), suggesting that a single TCR interacts with several different ligands. Variants of antigenic TCR ligands could be formed by modification of parts of the ligands, and these modified ligands induced several biochemical effects (James and Bevan, 1995).

TCR ligands can be classified into three different categones based on the responses of T cells. Ligands that cause T cell proliferation at low concentration are referred to as "agonists" (Rabinowitz *et al.*, 1996). Full activation is achieved when a TCR recognizes an

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agonistic peptide-MHC complex (Kersh *et al.*, 1996). Introduction of minor structural variation into agnonist ligands results in altered ligands; altered ligands that deliver only a part of the activation signal are referred to as "partial agonists", and those that block the TCR function are referred to as "antagonists" (Evavold and Allen, 1991). The binding and responses of T cells to altered ligands appears to govern the cross-reactive immune response and the cross-reactive responses of T cells to haptens such as arsonate (Nalefski and Rao, 1993), fluorescein (Siliciano *et al.*, 1986), and trinitrophenyl (Clayberger *et al.*, 1983) have been reported.

5D10.1B8, used in this study, is a murine T cell hybridoma which is specific for N-hydroxysuccinimidyl 4-azidobenzoate (HSAB) chemically conjugated with APC expressing class II MHC I-A^d (Thomas *et al.*, 1985). However, neither the binding site of HSAB on APC nor the mechanism of T cell stimulation by HSAB has been determined. Although it is expected that HSAB may bind to MHC molecules or MHC-associated peptide, the possibility of HSAB binding to other proteins on the surface of APC cannot be excluded. In this study, the role of class II MHC molecules in the recognition of HSAB by 5D10.1B8 T cell hybridoma was examined by using three different APCs expressing class II MHC proteins. In addition, HSAB-analogs were used to stimulate 5D10.1B8 to examine their partial agonistic or antagonistic properties.

Engagement of the TCR with ligand induces a series of signal transduction events critical for the functional activation of T cells including T cell development (Weiss *et al.*, 1994). The earliest detectable signaling event after TCR stimulation is the activation of protein tyrosine kinases, resulting in the tyrosine phosphorylation of TCR subunits and other cellular proteins (Weiss *et al.*, 1994). Here, we examined early TCR-dependent signals induced by HSAB and its analogs by the change of protein tyrosine phosphorylation pattern of the T cells upon stimulation.

Materials and Methods

Reagents

Dulbecco's Modified Eagle's Medium (DMEM), RPMI, NCTC-109, Hank' Balanced Salt Solution (HBSS), N-hydroxysuccinimidyl-4-azidobenzoate (HSAB), N-hydroxysuccinimidyl-4-azidosalicylate (HSAS), N-5-azido-2-nitrobenzoyloxysuccinimide (ANBS), and N-succinimidyl-6-(4'-azido-2'-nitrophenylamino) hexanoate (SANPAH) were purchased from Sigma Chemical Co (St. Louis, MO, U.S.A.). Fetal bovine serum (FBS) was from GIBCO (Detroit, MI, U.S.A.), and ^{3}H-thymidine was from Dupont (Boston, MO, U.S.A.). ECL Western Blotting reagent was purchased from Amersham Life Science (UK).

Cells

5D10.1B8 is a murine T cell hybridoma specific for HSAB chemically coupled to APCs expressing class II MHC protein I-A^d (Thomas *et al.*, 1985). A20.2JAD, LB27.4, and P388D1 were used as APC to stimulate 5D10.1B8 cells. A20.2JAD (H-2^d) and LB27.4 (H-$2^{d/b}$) are B cell lymphomas which express class II MHC, while P388D1 is a macrophage-like cell line which expresses class II MHC protein after 48 h incubation in the presence of 5% concanavalin A supernatant (CAS). CAS was a culture supernatant of rat spleen cells obtained after 24 h incubation in RPMI 1640 containing 10% FBS and 10 μg/ml concanavalin A (con A). The initial concentration of rat spleen cells was 1×10^7 cells/ml. APCs were maintained in RPMI 1640 containing 10% FBS, 5×10^{-5} M 2-mercaptoethanol, 2 mM L-glutamine, penicillin, and streptomycin, while 5D10.1B8 were cultured in DMEM containing 15% FBS, 5×10^{-5} M 2-mercaptoethanol, 2 mM L-glutamine, penicillin, streptomycin, 10% NCTC-109, and 50% HAT (hypoxanthine, aminopterin, thymidine).

Antibodies

Anti-MHC class II antibodies (anti-I-$A^{b,d}$ and I-$E^{d,k}$) were obtained from culture supernatant of M5/114.15.2 hybridoma cells. Fluorescein isothiocyanate (FITC)-labeled goat anti-rat IgG was purchased from Sigma Chemical Co. (St. Louis, Mo, U.S.A.), peroxidase-labeled anti-mouse IgG was purchased from Amersham Life Science (UK), and anti-phosphotyrosine (monoclonal $IgG2_{bk}$) was purchased from Upstate Biotechnology Inc (Lake Placid, NY, U.S.A.).

Stimulation of 5D10.1B8

APCs (A20.2JAD, P388D1, or LB27.4) were cultured in RPMI 1640 containing 5% CAS for 48 h unless otherwise indicated until logarithmic phase was reached, and were harvested by centrifugation at 300×g for 8 min. Each APC was washed three times with HBSS and suspended in 5 ml HBSS per 5×10^6 cells. While vortexing each APC suspension, 20 μl of HSAB, HSAS, ANBS, or SANPAH (stock solution of 10 mg/ml) was added to make 40 μg/ml final concentration. Each tube was wrapped with aluminum foil to protect from the light and was incubated at 37°C water bath for 1 h with occasional agitation, and subsequently each APC was washed 3 times with HBSS. 5D10.1B8 (1×10^5 cells/well) and each APC (1×10^5 cells/well) were plated on 96 well tissue culture plates and incubated in 5% CO_2 incubator at 37°C for 24 h. Culture supernatant (100 μl) from each well was collected and assayed for

interleukin-2 (IL-2) production.

IL-2 assay

CTLL-2 was cultured in RPMI 1640 containing 5% CAS, 10% FBS, 5×10^{-5} M 2-mercaptoethanol, 2 mM L-glutamine, penicillin/streptomycin for 2 days with an initial concentration of 2.5×10^4 cells/ml. IL-2-starved CTLL-2 cells were washed 3 times with HBSS and 4×10^3 cells (in 100 μl) per well were plated on 96-well plate. Cultured supernatant (100 μl) obtained from activated 5D10.1B8 cells was added to CTLL-2 and incubated for 16 h, and then ^{3}H-thymidine (0.5 μCi/well) was added and incubated for additional 12 h. Cells were harvested and the amount of ^{3}H-thymidine incorporated into the cells was counted using a scintillation counter.

Measurement of the level of class II MHC expression on APC by flow cytometer

To 1×10^6 cells of A20.2JAD, P388D1, or LB27.4 (CAS-induced or non-treated), 100 μl of M5/114.15.2 culture supernatant was added and incubated on ice for 40 min. Cells were washed 3 times with HBSS and incubated with 20 μl of FITC-labeled goat anti-rat IgG on ice for 40 min. After 3 washes with HBSS, cells were suspended in a small volume of HBSS and fixed with 1% para-formaldehyde followed by flow cytometric analysis.

Phosphotyrosine Immunoblot analysis

A20.2JAD were incubated with HSAB or its analogs for 1 h at 37°C as described above, and 1×10^6 cells were added in each well of 24-well tissue culture plate. 5D10.1B8 T cells (1×10^6/well) were added and, after a short spin at 1000 rpm for 1 min, incubated for indicated time periods at 37°C. After incubation, cells were harvested and lysed for 30 min on ice in lysis buffer containing 1% Triton X-100, 150 mM NaCl, 10 mM tris-HCl (pH 7.6), leupeptin (10 μg/ml), aprotinin (10 μg/ml), and 1 mM sodium orthovanadate. After removal of nuclear debris by centrifugation, the supernatants were analyzed by phosphotyrosine imunoblot analysis using 4G10, a mouse immunoglobin G2b (IgG2b) monoclonal antibody to phosphotyrosine, peroxidase-labeled anti-mouse IgG and ECL Western blotting reagents.

Results and Discussion

5D10.1B8 T cell hybridoma specifically recognizes a hapten, HSAB, which is chemically conjugated to APC expressing class II MHC, I-A^d (Thomas *et al.*, 1985). However, neither the exact binding site of HSAB on the surface of APC nor the mechanism of recognition by T cell is well characterized.

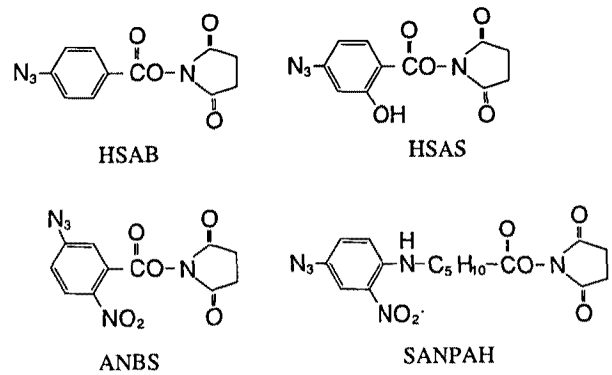

Fig. 1. Structures of HSAB and its analogs-HSAS, ANBS, and SANPAH.

As expected from the structure of HSAB shown in Fig. 1, N-hydroxysuccinimidyl group of HSAB can react with ε-amino group of proteins on the surface of APC, and HSAB-analogs will react in the same way. The stimulation of 5D10.1B8 by HSAB or its analogs is shown in Fig. 2. Three different APCs were used and the degree of stimulation of 5D10.1B8 was measured by the concentration of IL-2 secreted by 5D10.1B8. By comparing CAS-treated and non-treated APCs, the roles of class II MHC protein and accessory molecules were examined. When HSAB was used as antigen, CAS-induced APCs, i.e., A20.2JAD, P388D1, and LB 27.4, stimulated 5D10.1B8 more efficiently than non-treated ones and the most dramatic difference due to CAS treatment could be seen in the case of P388D1 (Fig. 2A, B, and C). The response of 5D10.1B8 to HSAB was greatest with CAS-treated LB27.4 (Fig. 2C). Several interpretations can be drawn from the result of Fig. 2. First, when P388D1 was used as APC, its antigen presenting ability was dramatically increased by CAS-treatment, showing that class II MHC proteins or other proteins induced by CAS containing IL-2 are essential for 5D10.1B8 stimulation. Second, even in the cases of A20.2JAD and LB27.4 that constitutively express class II MHC proteins, CAS treatment enhanced their stimulatory activity, indicating that increased expression of MHC protein by CAS might augment their HSAB-presenting abilities although other proteins induced by CAS might also be involved. Third, LB27.4 was the best APC in stimulating 5D10.1B8, suggesting that antigen-presenting abilities are different among APCs expressing same class II MHC proteins, which implies the importance of accessory molecules on the surface of APC.

In order to study the effect of the presence and expression level of class II MHC proteins on the antigen-presenting capabilities of APCs, quantitative analysis of

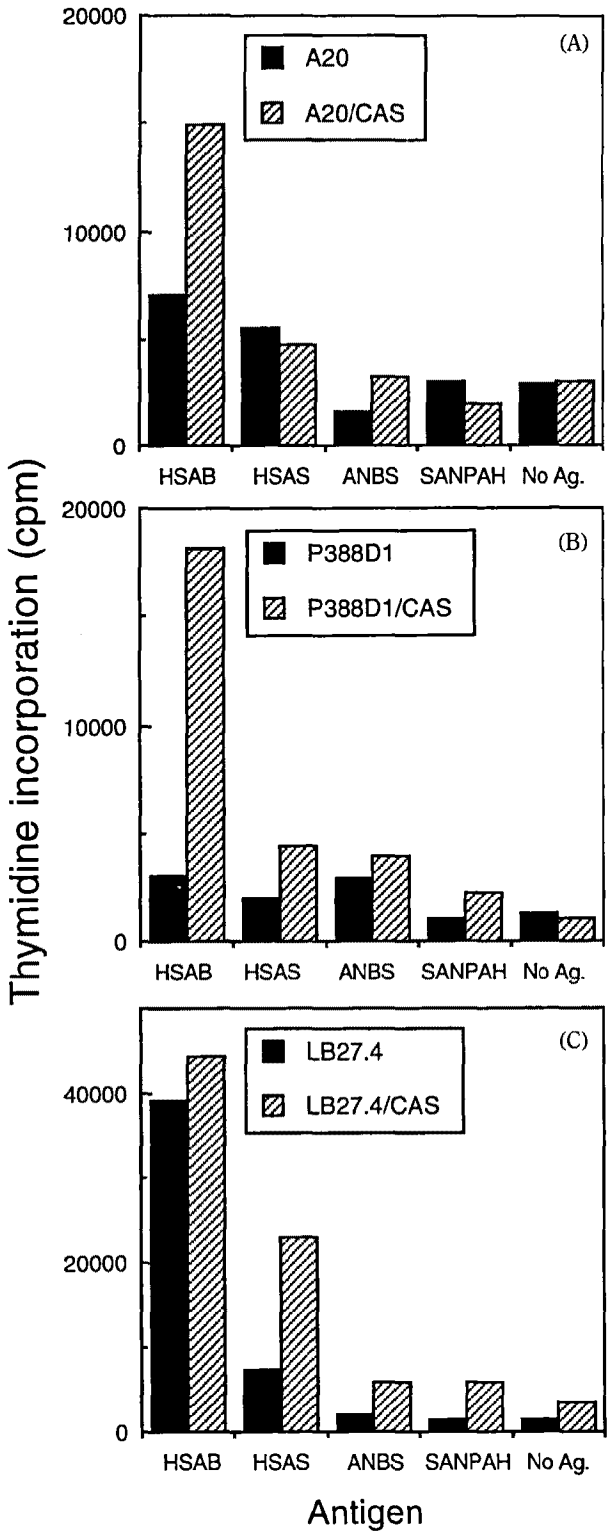

Fig. 2. Stimulation of 5D10.1B8 by HSAB and its analogs. 5D10.1B8 was stimulated by HSAB or its analogs-coupled A 20.2JAD (A), P388D1 (B), and LB27.4 (C). After 24 h, supernatants were obtained and IL-2 assay was performed. For each graph, in the right half, CAS-treated APC were used, and the left half is the result from non-treated APC. 1, 2, 3, 4, and 5 represent HSAB, HSAS, ANBS, SANPAH, and control (no antigen), respectively.

MHC protein expressed on the cell surface was performed by flow cytometry for CAS-induced or non-treated APCs. A20.2JAD and LB27.4 that express class II MHC constitutively showed increased expression of class II MHC after CAS treatment (Fig. 3B and F), consistent with the result of Fig. 2, in that, CAS-treatment enhanced the antigen-presenting abilities of APC. P 388D1 has been known to be induced to express class II MHC protein by IL-2 (Zlotnik *et al.*, 1983). In our case, however, very low level expression of class II MHC proteins on P388D1 was observed even after CAS treatment (Fig. 3C and D). Nevertheless, CAS-treated HSAB-conjugated P388D1 were able to stimulate 5D10.1B8 (Fig. 2B). One possible explanation is that macrophage-derived P388D1 cells use a different mechanism compare to the B cell lymphoma for antigen presentation (Kim *et al.*, 1985). Another possibility is that the low level expression of class II MHC might be enough to stimulate T cells, or that accessory molecules induced by CAS treatment were important for P388D1 to stimulate the T cells. Since hybridoma T cells do not usually require accessory molecules for activation, and thus artificial membranes containing MHC in the absence of other accessory molecules could successfully stimulate T cells (Watts and McConnell, 1986), the latter possibility might be of less importance. Collectively, results from Fig. 2 and Fig. 3 show that each APC ac-

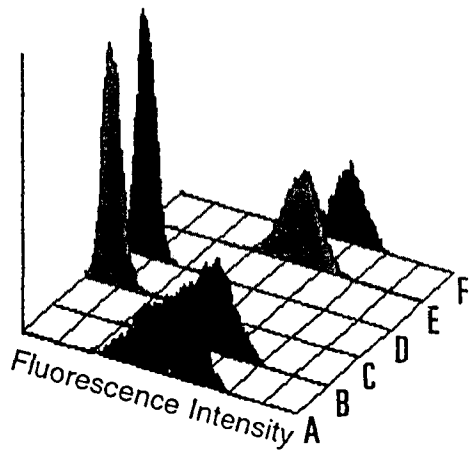

Fig. 3. Quantitative analysis of class II MHC proteins on A20. 2JAD, P388D1, and LB27.4 by flow cytometry. A20.2JAD (A and B), P388D1 (C and D), and LB27.4 (E and F) with CAS treatment (B, D, and F) or no treatment (A, C, and E) were stained with anti-MHC class II antibody (M5/114.15.2 culture supernatant) and subsequently with FITC-labeled goat anti-rat IgG, followed by flow cytometrical analysis.

tivated T cells differentially, also CAS augmented the antigen presenting ability of all APCs, suggesting that increased expression of class II MHC protein by CAS containing IL-2 played an important role in HSAB presentation and T cell activation.

To prove the role of I-A^d in stimulation of 5D10.1B8, we attempted to block the 5D10.1B8 activation by using anti-I-A^d antibody. When A20.2JAD and LB27.4 were used as APCs, anti-I-A^d antibody inhibited the activation of 5D10.1B8 almost completely, and anti I-A^b or anti-HLA-A2 antibody showed little effect (Fig. 4), indicating that stimulation of 5D10.1B8 by HSAB was I-A^d-dependent. However, when P388D1 was used as APC, the inhibition of 5D10.1B8 activation by anti-I-A^d antibody was only 42%, suggesting the possibility that contribution of accessory molecules might be significant for P388D1 to activate 5D10.1B8. The APCs used in this experiment were treated with CAS for 68 h rather than 48 h (as for the experiments shown in Fig. 2B and C), and it should be noted that the stimulating capability of P388D1 was comparable to that of LB27.4 (Fig. 4), while the expression level of I-A^d showed little difference between P388D1 treated with CAS for 68 h and that treated for 48 h (data not shown). These results again suggest the possibility of an important role for accessory molecules induced by CAS treatment.

Binding of TCR to peptide-MHC triggers a series of intracellular biochemical reactions in the T cell, such as acid release, tyrosine phosphorylation of TCR subunits, calcium flux, and T cell proliferation (Rabinowitz *et al*, 1996). The early events, occurring within seconds, include tyrosine phosphorylation of the TCR-associated ζ proteins of CD3 molecule (Rabinowitz *et al.*, 1996), resulting in subsequent tyrosine phosphorylation of various cellular proteins and activation of the phosphatidyl inositol pathway (Rabinowitz *et al.*, 1996). Since HSAB could stimulate 5D10.1B8 more efficiently than HSAS, it would be necessary to see if the signaling events triggered by the two antigens were different. Thus, the protein tyrosine phosphorylation pattern of 5D10.1B8 was examined after its stimulation (Fig. 5A). Tyrosine phosphorylation level of a protein of 18-20 kD in 5D10.1B8 appeared to increase after 2 h incubation with HSAB-treated APC (Fig. 5A). When HSAB-analogs were used to stimulate 5D10.1B8, HSAS also appeared to induce tyrosine phosphorylation of the 18-20 kD protein (Fig. 5B), presumably the CD3-ζ chain (Madrenas *et al.*, 1995), whereas other analogs did not. The facts that HSAB and HSAS induced similar protein tyrosine phosphorylation patterns (Fig. 5B) and that HSAS stimulated only 50% IL-2 release compared to HSAB (Fig. 2C) suggest that the biochemical events between protein tyrosine phosphorylation and IL-2 release may be different for HSAB- and HSAS-stimulated T cells.

When the responses of 5D10.1B8 to HSAB-analogs were examined, LB27.4-coupled HSAS stimulated 5D 10.1B8 significantly, and the stimulation by HSAS was about 50% of that by HSAB (Fig. 2C), suggesting that HSAS has partial agonistic properties when presented by LB27.4. Other analogs, however, did not appear to induce IL-2 secretion from 5D10.1B8, although the possibility that they caused some biochemical changes with-

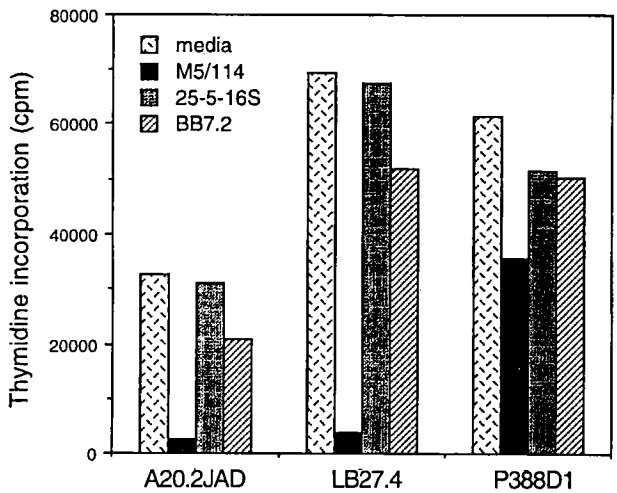

Fig. 4. Blocking of 5D10.1B8 stimulation by HSAB using anti MHC class II antibody. A20.2JAD, P388D1, and LB27.4 were treated with 5% CAS for 68 h, followed by incubation with HSAB. Stimulation of 5D10.1B8 by HSAB was performed in the presence of 20% anti-MHC class II antibody (M5/114.15.2). As a negative conrol, 20% of anti-I-A^b (25-5-16S) or anti-HLA-A2 (BB7.2) was used.

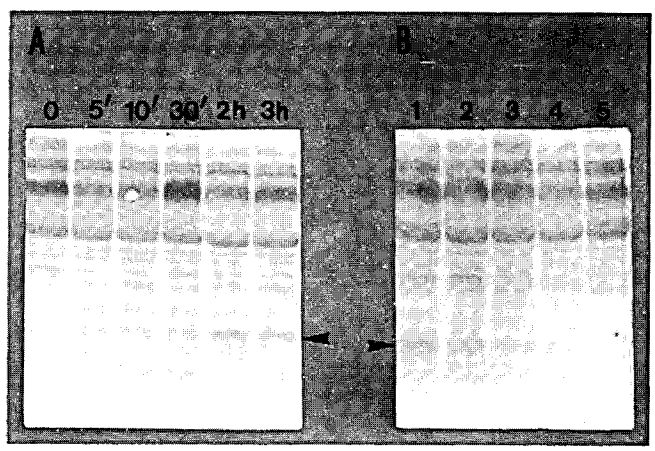

Fig. 5. Phosphotyrosine Immunoblot analysis of activated 5D 10.1B8. Stimulation and processing of 5D10.1B8 were performed as described in Materials and Methods. Time-dependent protein tyrosine phosphorylation pattern of 5D10.1B8 is shown in panel A. The arrowhead indicates a protein whose tyrosine phosphorylation level was increased. 5D10.1B8 was stimulated with HSAB and its analogs for 2 h. In panel B, 1, 2, 3, and 4 indicate HSAB, HSAS, ANBS, and SANPAH, respectively.

in the cells may not be excluded.

The antigen processing mechanism by which APC generates antigenic determinants from foreign antigen can occur in two ways depending on the nature of antigen. For large protein antigens, uptake by APC and antigen processing steps which involves lysosomal denaturation and proteolytic cleavage of antigen are usually required (Ziegler and Unanue, 1982; Allen *et al.*, 1984). In this case, APCs fixed with glutaraldehyde or paraformaldehyde and APCs treated with agents that inhibit lysosomal proteolysis fail to form proper antigenic determinants (Allen *et al.*, 1984). For samller peptide antigens or haptens, antigen processing does not seem to be required (Allen *et al.*, 1984), and antigen associated with MHC on the surface of APCs can be recognized by T cells (Allen *et al.*, 1984), and therefore, fixed APCs would be able to present antigen as efficiently as untreated ones. Hence, the presenting pathway of HSAB or its analogs might be operating in two possible ways. First, haptenic antigen can be conjugated directly to MHC protein on the surface of APC without any other processing and recognized by TCR. Second the haptenic antigens conjugated to certain surface molecules are internalized and processed like large protein antigens before they are complexed with MHC to be presented to T cells, although it may not be excluded that the requirement of antigen processing could be different for each APC. These two possibilities are yet to be examined.

Furthermore, the azido group of HSAB can be activated by light and conjugated to protein by photolysis (Thomas *et al.*, 1985). If HSAB-conjugated APC and T cells are exposed to ultraviolet light, the crosslinking of APC-HSAB-T cell might occur by the binding of azido group of HSAB to a part of the TCR. Therefore, 5D10. 1B8 provides a useful system in the elucidation of the binding sites of haptenic antigen on the APCs and the TCR region which recognize haptens such as HSAB.

References

Allen, A., Beller, D. I., Braun, J. and Unanue, E. R. (1984) *J. Immunol.* **132**, 323.

Cavani, A., Hackett, C. J., Wilson, K. J., Rothbard, J. B. and Katz, S. I. (1995) *J. Immunol.* **154**, 1232.

Clayberger, C., Dekruyff, R. H., Aisenberg, J. and Cantor, H. (1983) *J. Exp. Med.* **157**, 1906.

Crabtree, G. R. (1989) *Science* **243**, 355.

Eisen, H. N., Orris, L. and Belman, S. (1952) *J. Exp. Med.* **95**, 473.

Evavold, B. D. and Allen, P. M. (1991) *Science* **252**, 1308.

Jameson, S. C. and Bevan, M. J. (1995) *Immunity* **2**, 1.

Kersh, G. J. and Allen, P. M. (1996) *Nature* **380**, 495.

Kim, K., Solvay, M. and Thomas, D. W. (1985) *Cell. Immunol.* **96**, 267.

Kohler, J., Hartmann, U., Grimm, R., Pflughelder, U. and Weltzien, H. U. (1997) *J. Immunol.* **158**, 591.

Little, J. R. and Eisen, H. N. (1996) *Biochemistry* **5**, 3385.

Madrenas, J., Wange, R. L., Isakov, N., Samelson, L. and Germain, R. N. (1995) *Science* **267**, 515.

Mueller, D. L., Jenkins, M. K. and Schwartz, R. H. (1989) *Annu. Rev. Immunol.* **7**, 445.

Nalefski, E. A. and Rao, A. (1993) *J. Immunol.* **159**, 3806.

Pohlit, H., Hass, W. and Boehmer, H. von (1979) *Eur. J. Immunol.* **9**, 681.

Rabinowitz, J. D., Beeson, C., Wulfing, C., Tate, K., Allen, P. M., Davis, M. M. and McConnell, H. M. (1996) *Immunity* **5**, 125.

Sette, A., Alexander, J., Ruppert, J., Snoke, D., France, A., Ishioka, G., and Greg, H. M. (1994) *Annu. Rev. Immunol.* **12**, 413.

Shearer, G. M. (1974) *Eur. J. Immunol.* **4**, 527.

Siliciano, R. F., Hemesath, T. J., Pratt, J. C., Dintzis, R. Z., Dintzis, H. M., Acuto, O., Shin, H. S., and Reinherz, E. L. (1986) *Cell* **47**, 161.

Thomas, D. W., Eades, L., Wilson, C. and Solvay, M. J. (1985) *Cell. Immunol.* **96**, 351.

Watts, T. H. and McConnell, H. M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9660.

Weiss, A. and Littman, D. R. (1994) *Cell* **76**, 263.

Weltzien, H. U., Moulon, C., Martin, S., Padovan, E., Hartmann, U. and Kohler, J. (1996) *Toxicology* **107**, 141.

Ziegler, H. K. and Unanue, E. R. (1982) *Proc. Natl. Sci. USA* **79**, 175.

Zlotnik, A., Shimonkevitz, R. P., Gefter, M. L., Kappler, J. and Marrack, P (1983) *J. Immunol.* **131**, 2814.