Tween-20 in Tris-buffered saline, the membrane was incubated with the rabbit anti-Syk Ab, washed extensively and subjected to chemiluminescence detection with peroxidase-conjugated anti-rabbit IgG Ab (Santa Cruz Biotechnology, Inc.), using an ECL kit (Amersham).

Anergy induction assay

SF36.16 T cells were primarily stimulated with soluble-form wild-type peptide (1 μM BCGap84-100), immobilized anti-DR mAb, immobilized anti-CD3 mAb or irradiated autologous PBMC prepulsed with the wild-type peptide (5μM BCGap84-100 for 5h at 37 °C) in 24-well flat-bottom culture plates. Seven days later, these T cells were washed with culture medium and co-cultured with irradiated autologous PBMC in the presence of various concentrations of wild-type BCGap84-100. The T cells were cultured in a 96-well plate for 72 h, and subjected to [^{3}H]thymidine incorporation assay.

Fibroblasts

The human PDL used in this study was isolated from two periodontally healthy donors carrying DRB1*1501-DQB1*0602-DPB1*0501 / DRB1*0405-DQB1*0401-DPB1*1901, and DRB1*1302-DQB1*0604-DPB1*0301 / DRB1*0901-DQB1*0303-DPB1*0401 haplotypes. PDL were maintained in a medium consisting of Dulbecco's Modified Eagle's Medium (DMEM; Life Technologies) supplemented with 10% fetal bovine serum (FBS; Irvine Scientific, Santa Ana, CA, USA), 2 mM glutamine, 50 μg/ml gentamicin, 0.2 mM non-essential amino acids (all additives were from Life Technologies) at 37℃ in 95% air and 5% CO_2. All experiments were carried out while these cells were actively growing between passages 3-8. The expression of HLA-II molecules on fibroblasts treated with or without IFN-γ was evaluated using flow cytometry with Epics (Beckman Coulter, Fullerton, CA).

RESULTS

Monokine production induced by anti-HLA mAbs

We examined the monokine secretion induced by cross-linking class II HLA molecules, using solid-phase mAbs to class II HLA, by which involvement of cell-surface molecules other than HLA is unlikely to occur. As shown in Fig. 1A, the effect of the anti-DQ

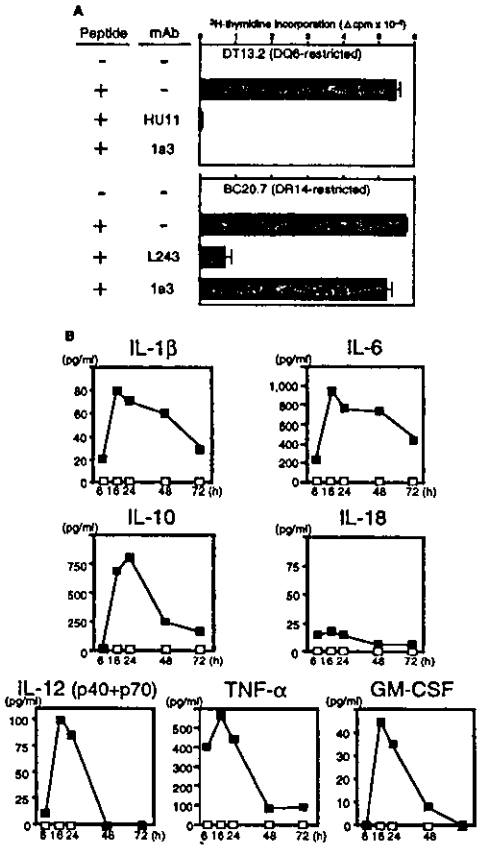

Figure 1. Monokine secretion induced by solid-phase mAbs to HLA. (A) DT13.2 and BC20.7 were cultured in the presence of *Der f* 1 peptide (for DT13.2) or BCGa peptide (for BC20.7) and irradiated autologous PBMC, with or without anti-class II HLA mAbs. (B) Adherent cells were incubated at 6 x 10^4 cells/well where 10 μg/ml of 1a3 (closed square) and mouse IgG2a (open square) are immobilized, at 37 °C in a CO_2 incubater. Culture supernatants were collected at the indicated time points.

mAb should be specific, because liquid-phase 1a3 (simple co-culture) did abrogate HLA-DQ-restricted T-cell clonal responses (DT13.2), but not HLA-DR-restricted responses (BC20.7). However, solid-phase 1a3 markedly stimulated monocytes to produce IL-1β, IL-6, IL-10, IL-12 (p40 + p70), TNF-α and GM-CSF, whereas Ig subclass-matched control (mouse IgG2a) did not, as shown in Fig. 1B.

Activation of MAP kinases by anti-HLA mAbs

We next examined the effects of various inhibitors for signal transduction molecules. As shown in Fig. 2A, PD98059 (MEK-1 inhibitor) and SB203580 (p38 inhibitor) inhibited anti-DR-induced IL-1β production from monocytes. Genistein exhibited bi-phasic effect and inhibited IL-1β production at high concentrations (500 μM). We then studied the phosphorylation of various kinases by cross-linking class II HLA, among which only MAP kinases exhibited differential activation by anti-DR, -DQ and -DP. We stimulated monocytes directly with solid-phase anti-HLA mAbs, and cell lysates were subjected to Western blot analysis, using Abs to phosphorylated forms of Erk, JNK and p38 (anti-pErk, anti-pJNK and anti-pp38, respectively). As shown in Fig. 2B and 2C, Erk, especially Erk2, was phosphorylated only by anti-DR mAb (very weak phosphorylation was detected by anti-DQ or anti-DP, in the original film), whereas p38 was phosphorylated by anti-DR, anti-DQ and anti-DP mAbs.

Induction of monokine secretion from peptide-pulsed monocytes, using emetine-treated T cells of various HLA-restriction patterns

We wanted to determine if natural TCR-peptide-HLA interactions would induce monokine secretion by signaling through class II HLA molecules. T cell clones of various HLA-restriction patterns were treated with *de novo* protein synthesis inhibitor emetine. This is because it is highly likely that T-cell membrane proteins or T-cell soluble factors newly synthesized after activation, work in turn on monocytes. As shown in Table 1, three human Th0 clones of distinct HLA restriction patterns, BC20.7 (BCGa-specific, DR14-restricted), DT13.2 (*Der f* I-specific. DQ6-restricted) and OT1.1 (p53-specific, DP5-restricted), were used for emetine-treatment, seven days after the last antigenic stimulation. First, we determined the ED50 of each clone to be 0.008 μM, 0.18 μM and 0.10 μM, for BC20.7, DT13.2 and OT1.1, respectively (not shown). Monocytes were pulsed with peptides, the concentrations of which were 625-fold as much as the ED50 (5 μM, 112.5 μM and 62.5 μM, for BC20.7, DT13.2 and OT1.1, respectively), followed by co-culture with emetine-treated T cells. These peptide concentrations induced plateau responses of monokine. Peptide-pulsed monocytes co-cultured with emetine-treated T cells, as shown in Table 1, produced IL-1β, IL-6, IL-10, IL-12 (p40 + p70), GM-CSF and TNF-α. It is noteworthy that the DR14-restricted clone, BC20.7, tends to induce pro-inflammatory monokines, such as IL-1β (105 pg/ml) and TNF-α (887 pg/ml) with the IL-10 / IL-1β ratio being 1.6, whereas DQ6-restricted clone, DT13.2, and DP5-restricted clone, OT1.1, tend to induce anti-inflammatory monokine IL-10 (787 pg/ml and 725 pg/ml, respectively) with the IL-10 / IL-1β ratio being 32.8 and 34.5 for DT13.2 and OT1.1, respectively. Allogeneic monocytes that do not share restriction HLA molecules, exhibited marginal monokine production in the presence of emetine-treated T cells.

Effects of protein kinase inhibitors on monokine productions

We co-cultured peptide-pulsed monocytes and emetine-treated BC20.7 T cells in the presence of several kinase inhibitors. These inhibitors were dissolved in DMSO and added to the culture medium at a final content of 0.5%, a content which did not inhibit DR-mediated monokine production. All data (not shown) collectively suggest that: (*a*) p38 is involved in both IL-1β and IL-10 production induced by ligating DR molecules expressed on monocytes; (*b*) MEK-1-Erk pathway is only partially involved in IL-1β production, being independent from p38-associated IL-1β

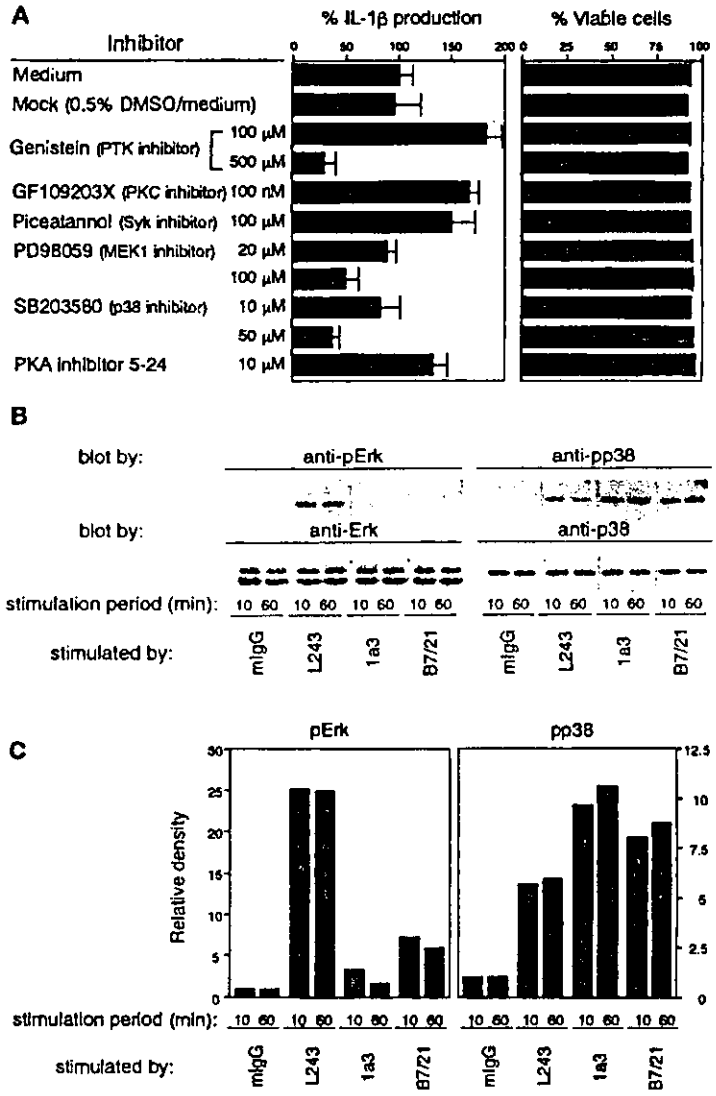

Figure 2. Activation of MAP kinase by mAbs to class II HLA. (A) Monocytes were co-cultured for 16 h on anti-class II coated plates, with the indicated inhibitors, at the indicated concentrations. Culture supernatants were collected and stored in aliquots at -80°C until determinations of cytokine concentrations. One hundred % IL-1β production was 205 pg/ml. Viable cell contents were determined using trypan blue. (B) After 10 and 60 min of stimulation with solid-phase mAbs, monocytes were lysed in 50 μl of lysing buffer, which were subjected to Western blot analysis either with Abs specific for Erk and p38, or with activated form of Erk and p38. (C) Relative densities are shown based on B.

Table 1. Monokine production from monocytes stimulated with emetine-treated T cells + peptide.

Monokine	T cell clones for stimulation BC20.7	DT13.2	OT1.1
IL-1β	105.0 (pg/ml)	24.0	21.0
IL-6	87.5	62.5	140.0
IL-10	172.5	787.5	725.0
IL-18	<15.0	<15.0	<15.0
IL-12 (p40 + p70)	145.0	75.0	20.5
GM-CSF	475.0	1150.0	887.5
TNF-α	887.5	642.5	230.0
IL-10 / IL-1β	1.6	32.8	34.5

Emetine-treated T cells (BC20.7, DT13.2 and OT1.1) were cultured with peptide-prepulsed monocytes. The concentration of the peptides for each clonal responses was 625-fold as much as the ED50 (5 μM, 112.5 μM and 62.5 μM for BC20.7, DT13.2 and OT1.1, respectively). Culture supernatants after 16- (for IL-12), 24- (for IL-1β, IL-10, IL-18, GM-CSF and TNF-α) and 48-h (for IL-6) incubation were collected, and subjected to ELISA. Results are expressed as the mean value of triplicate determinations. Standard error was less than 20%.

production; and (*c*) activation of Erk may inhibit p38-mediated IL-10 production (Fig. 3).

Restriction molecules and cytokine-production patterns of short-term T cell lines

If the phenomenon observed earlier in this study occurs in a local milieu of T cell differentiation, lymphokine production patterns of T cells would be affected by restriction HLA molecules. Then, we next examined the production of IFN-γ and IL-4 from the *Der f*-specific T cell lines. As shown in Figure 4A, DR-restricted T cell lines produced more IFN-γ than IL-4, but DP-restricted T cell lines did more IL-4 than IFN-γ (p = 0.02 and 0.04 in donor MA and NI, respectively). MAbs used in this study did not induce monokine secretion, when used as a soluble form (not shown). HLA types of MA (HLA-DRB1*1502-DRB5*0102-DQA1*0103-DQB1*0601 / HLA-DRB1*1405-DRB3*0202-DQA1*0101DQB1*0503) and NI (HLA-DRB1*0901-DRB4*0101-DQA1*0301-(HLA-DRB1*0901-DRB4*0101-DQA1*0301-DQB1*0303 / HLA-DRB1*1302-DRB3*0301-DQA1*0102-DQB1*0605) were distinct. Then, we examined the production of IFN-γ and IL-4 from the PPD-specific T cell lines (Fig. 4B). DR-restricted T cell lines produced more IFN-γ than IL-4, but DQ-resricted lines did more IL-4 than IFN-γ (p = 0.04 in donor MA). We next used X19 (random 19-mer peptide) to confirm

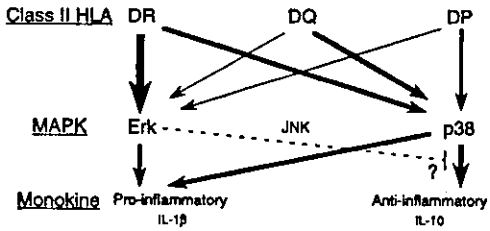

Figure 3. Summary of class II HLA-mediated MAPK activation. Note that other class II signaling elements that can be additive or modify the signaling via MAP kinases are not illustrated.

the phenomenon observed in earlier studies, because: (*a*) DQ-restricted / *Der f*-reactive and DP-restricted / PPD-reactive T cells were not readily established; and (*b*) X19 can stimulate most $CD4^+$ memory T cells to proliferate in the presence of cytokines, under cloned conditions. Indeed, DR-, DQ-, and DP-restricted T cell lines were obtained, the cytokine profiles of which again exhibited the similar results (Fig. 4C). Moreover, we titrated down the peptide concentration for DR-restricted responses and found that lower concentrations of X19 peptide did not lead to DQ/DP-restricted patterns of cytokines (not shown), which was indeed the case when emetine-treated BC20.7 T cells were incubated with monocytes in the presence of lower concentration of the antigenic peptide (not shown). These data indicate that DR-restricted and already activated peripheral $CD4^+$ T cells carry Th1-prone phenotype, compared with DQ- / DP-restricted T cells, although the segregation pattern is incomplete.

Cross-linking HLA-DR molecules on B cells induces increased production of IgM without inducing B-cell proliferation.

To test whether signals via class II HLA molecules would affect production of Igs, we first cross-linked class II HLA molecules on B cells by making use of anti-DR mAb-coated culture plates (21). The supernatant fluids of 5-day cultures were assayed for Ig concentrations, among which only IgM was markedly affected

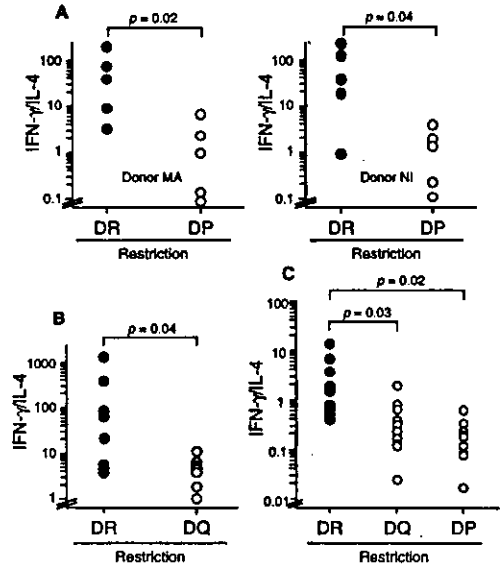

Figure 4. Restriction molecules and cytokine-production patterns of short-term T cell lines. *Der f* (crude mite antigen)-specific short-term T cell lines (A), PPD-specific short-term T-cell lines (B) and X19-reactive T cell clones (C) of various restriction patterns were restimulated with excess concentrations of antigens (A and B: 10 μg/ml, C: 500 μM), then after 48-h incubation, culture supernatants were collected for measurements of IFN-γ and IL-4 production by ELISA. One spot indicates one cell line.

by DR ligation. As shown in Fig. 5A, cross-linking DR molecules with anti-DR mAbs (L243 or HU4) on B cells induced IgM production, whereas isotype-matched mouse IgG did not do so, thereby indicating that signals transmitted by FcR are not involved. Similar results were obtained, using B cells from another subject carrying DRB1*1405/1502 (data not shown).

Cross-linking HLA-DR molecules enhances both membrane-type and secretory-type IgM heavy chain gene expression.

To determine whether signals via DR molecules up-regulate μ chain mRNA, we cross-linked DR molecules on peripheral B cells (1×10^6) with either solid-phase anti-DR mAb

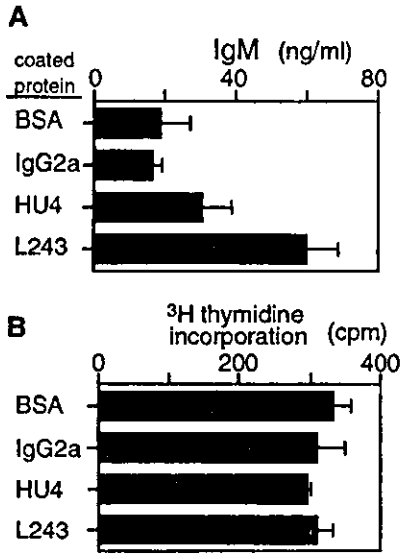

Figure 5. Cross-linking HLA-DR molecules on B cells induces increased production of IgM (*A*), without inducing proliferation (*B*). Mouse IgG2a, anti-DR mAb HU4, anti-DR mAb L243, or BSA were coated onto 96-well flat-bottomed culture plates at 10 μg / ml PBS. Purified peripheral B cells were incubated at 5 x 10^4 cells/well where mAbs are coated, at 37 °C in a CO_2 incubator for 3 days (proliferation assay) or for 5 days (IgM determination). HLA type of the B-cell donor was DRB1*0101/1201. Mean cpm of triplicate responses ± SD is indicated.

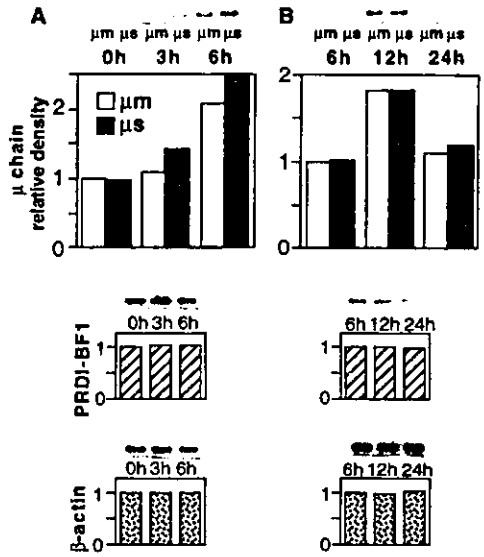

Figure 6. Cross-linking DR molecules enhances μ chain mRNA expression. Purified peripheral B cells were incubated in 24-well flat-bottomed culture plates at 1 x 10^6 cells/well where mAbs are coated, at 37 °C in a CO_2 incubator for 0, 3 and 6 h (*A*) or 6, 12 and 24h (*B*). As described under *experimental procedures*, RT-PCR and Southern blot analysis were done for membrane-type μ chains (μm; open columns), secretory-type μ chains (μs; closed columns), PRDI-BFI (hatched columns) and β–actins (shaded columns). mRNA expression levels were quantified using NIH image and represented by relative values compared with those from 0-h membrane-type μ chain, 0-h PRDI-BF1, or 0-h β–actin (A) and 6-h membrane-type μ chain, 6-h PRDI-BF1, or 6-h β–actin (B). HLA type of the B-cell donor was DRB1*0101/1201.

(L243) or solid-phase mouse IgG. Due to limitations in the number of purified B cells, we could test only 3 samples at one time. At 0, 3 and 6 h (Fig. 6A), or 6, 12 and 24 h (Fig. 6B) after the initiation of culture, B cells were analyzed for mRNA expression for μ chains, using RT-PCR and Southern blot analysis. Relative mRNA level was analyzed. When we tested the kinetics, μ chain mRNA increased in a time-dependent fashion (Fig. 6A), and reached maximum at 12 h (Fig. 6B). This increase was not due to the enhanced recovery of mRNA, as evidenced by the presence of an equal amount of β-actin mRNA in each sample. The μ chain mRNA level induced by control mouse IgG at 3, 6, 12 and 24 h was practically the same as that induced by anti-DR mAbs at 0 h (data not shown).

To test whether the DR-generated signal induced differentiation of B cells to plasma cells, we analyzed PRDI-BF1 transcripts. PRDI-BF1 is a human homologue of Blimp-1, the expression of which is characteristic of late B cells and plasma cells (22,23). However, as shown in Fig. 6, DR-generated signals up-regulated no mRNA for PRDI-BF1. The presence of PRDI-BF1 transcripts is indicative of the presence of plasma cells in this cell preparation. These data suggest that IgM production induced by cross-linking of DR molecules is regulated at the mRNA level, and is not associated with B-cell differentiation to plasma cells.

Emetine-treated and HLA-DR-restricted T cells are capable of inducing IgM production by B cells

Although earlier observations strongly suggest that the ligation of HLA-DR molecules directly stimulates B cells to produce IgM, the outcome of ligation by mAbs should be affected by epitopes recognized by these mAbs and their affinity. Indeed, anti-HLA-DR mAb HU-4, exerted weaker effects than did L243 (Fig. 5). It is unlikely that HLA-DRB4 molecules recognized by L243 are transmitting the signals, because the B-cell donor in Figures 5 and 6 did not carry *DRB4*-positive haplotypes. Therefore, we next asked if a similar phenomenon occurs, on natural TCR-peptide-HLA interactions. An HLA-DR-restricted T cell clone was treated with the *de novo* protein synthesis inhibitor emetine, because it is highly likely that T-cell membrane proteins or T-cell soluble factors newly synthesized after activation by peptide-pulsed B cells, work on B cells. Under conditions where T cells are treated with 90 μg / ml emetine for 1 h followed by co-culture with peptide-pulsed B cells bearing restriction HLA molecules, T cells produced <25 pg / ml of IL-4, whereas non-treated T cells produced 3580 pg / ml of IL-4, although cell-surface TCR remains practically the same level (data not shown), indicating that *de novo* protein synthesis of T cells is abrogated by emetine. A T-cell clone BC20.7 (BCGa-specific, DR14-restricted) and B cells purified from PBMC of the donor of BC20.7, was used in subsequent experiments. As shown in Table 2, levels of IgM, IgG1, IgG4, IgE, and IgA were detected when mock-pulsed B cells were co-cultured with emetine-treated T cells. However, when B cells were pre-pulsed with the antigenic peptide, marked enhancement of IgM and marginal enhancement of IgA production were observed and such was not the case when peptide-pulsed B cells were cultured in the absence of T cells (not shown).

Cross-linking DR molecules on B cells up-regulates Syk kinase activity

To investigate possible protein tyrosine phosphorylation associated with this event, detergent lysates of peripheral B cells and LD2B cells treated with anti-DR mAb or control mouse IgG, were analyzed. Fig. 7 shows that protein-tyrosine phosphorylation was enhanced by cross-linking of DR molecules on peripheral B cells (Fig.7A, *lane* 2 vs 1, 3). Bands corresponding to proteins with an approximate molecular mass of 65, 70, 110 and

Table 2. *Ig production from B cells induced by a DR-restricted T-cell clone.*

peptide	IgM	IgG1	IgG2	IgG3	IgG4	IgE	IgA
	ng / ml						
-	37.5	124.5	<3.1	<3.1	3.13	31.2	480
+	223.5	96.0	<3.1	<3.1	1.41	21.2	980

B cells either mock-pulsed or pulsed with BCGap84-100 were cultured with an HLA-DR14 (DRB1*1405)-restricted and emetine-treated T cell clone BC20.7 for 5 days. B cells were purified by, and the T-cell clone was established from a donor carrying DRB1*1405/1502. Mean values of duplicate determinations are indicated. SD was less than 25%.

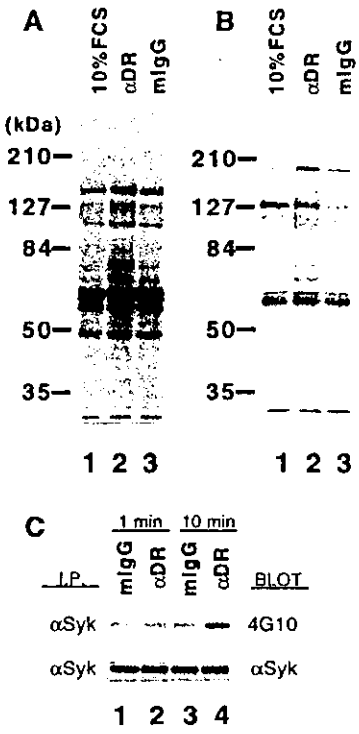

Figure 7. Cross-linking DR up-regulates protein-tyrosine phosphorylation of Syk. The peripheral B cells (*A*) and LD2B B cells (*B* and *C*) were incubated with biotinylated anti-DR mAb L243 or biotinylated mouse IgG followed by avidin for 10 min (*A* and *B*) or for 1 and 10 min (*C*), then cells were lysed. Cell lysates were either immunoprecipitated by anti-Syk Ab (*C*) or directly (*A* and *B*) resolved by 9 % SDS-PAGE, and then transferred to nitrocellulose membranes. Protein-tyrosine phosphorylation was detected by using anti-phosphotyrosine mAb (4G10) and ECL.

130 kDa were reproducibly hyper-phosphorylated. Likewise, cross-linking of DR molecules on LD2B induced tyrosine-phosphorylation of 65 and 70 kDa proteins (Fig. 7B, *lane* 2 vs 1, 3). Furthermore, immunoprecipitation by anti-Syk Ab followed by blotting with anti-phosphotyrosine mAb 4G10, exhibited anti-DR-induced tyrosine phosphorylation of Syk molecules expressed in LD2B cells (Fig. 7C).

To further confirm that Syk is activated directly via HLA-DR, Syk kinase activity was determined by in vitro kinase assay, using Syk molecules immunoprecipitated with anti-Syk Ab, and MBP as a substrate. Because a large number of B cells are required for immunoprecipitation followed by in vitro Syk kinase assay, we used a B lymphoblastoid cell line LD2B homozygous for DRB1*1501, which secretes IgM in the absence of specific stimuli. LD2B was selected among many B cell lines because (*a*) anti-DR-induced phosphorylation pattern of LD2B was similar to that of peripheral B cells, including the phosphorylation of 70-kDa protein (Fig. 7); and (*b*) LD2B B cells expressed IgM heavy chain genes. Enhancement of IgM production from LD2B B cells after cross-linking DR molecules was only marginal, probably because LD2B cells constitutively showed a 50- to 80-fold higher IgM secretion than did peripheral B cells, on a single cell basis (data not shown). As shown in Fig. 8A, however, cross-linking of DR molecules by biotinylated anti-DR mAb plus avidin, induced marked phosphorylation of MBP (*lane* 3), whereas incubation of LD2B with biotinylated mouse IgG + avidin, only marginally induced phosphorylation of MBP (*lane* 2 vs 1). Because MBP is not a substrate specific for Syk kinase, and it might be that MBP was phosphorylated by certain kinases co-precipitating with Syk, we also asked if the effect of Syk on MBP would be inhibited competitively by HS1p388-402 peptide, a substrate specific for Syk. In vitro Syk kinase assay with MBP (2 μg/sample; 0.11 nmol/sample) was done in the presence of either a 250-fold molar excess of the HS1 peptide (27.5 nmol/sample) or an irrelevant peptide carrying two tyrosine residues (EIKYNGEEYLIL; 27.5 nmol/sample). Indeed, MBP phosphorylation was inhibited by the HS1 peptide, but not by the irrelevant peptide (*lanes* 4 and 5).

It is also important to note that Syk molecules are associated with FcγR and I are activated by cross-linking of the receptor (24,25). It is therefore conceivable that the increment in Syk kinase activity we observed

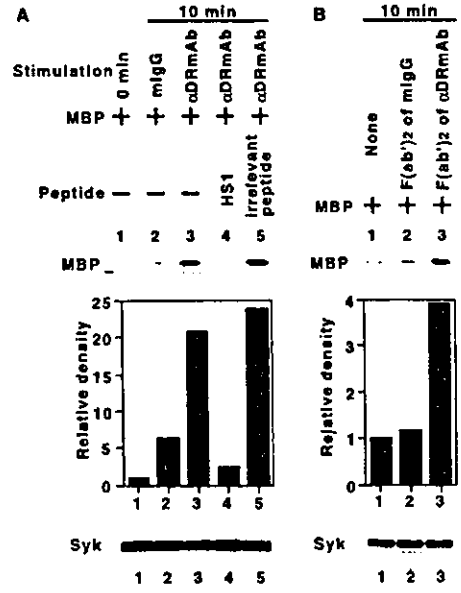

Figure 8. Cross-linking DR up-regulates Syk kinase activity. LD2B B cells were incubated with biotinylated anti-DR mAb L243 or biotinylated mouse IgG followed by avidin for 10 min, then cells were lysed. Lysates were immunoprecipitated with anti-Syk Ab. (*A*) An aliquot of immunoprecipitated proteins were immunoblotted with anti-Syk Ab. Residual Syk proteins on agarose beads were used for in vitro immune complex kinase assay, using MBP as a substrate. The HS1 peptide or an irrelevant peptide was added to the assay. (*B*) LD2B B cells were incubated with biotinylated F(ab')2 of anti-DR mAb L243, or biotinylated F(ab')2 of control mouse IgG followed by avidin for 10 min. Cells were lysed, immunoprecipitated, and subjected to in vitro Syk kinase assay. MBP phosphorylation levels were quantified using a bio-imaging analyzer (BAS2000, Fuji Film, Tokyo), and represented by relative values compared with those of 0 min (*A*) or 10 min (*B*) (unstimulated cells).

may be due to cross-reaction of mouse Ig with human FcγR expressed on B cells. To exclude this possibility, we prepared a biotinylated F(ab')2 fragment of anti-DR mAb L243 or that of control mouse Ig. As shown in Fig. 8B, cross-linking of F(ab')2 fragment of anti-DR mAb L243 induced phosphorylation of MBP (*lane* 3), whereas F(ab')2 fragment of mouse Ig induced little phosphorylation of MBP (*lane* 2), compared with a control (avidin only; *lane* 1). This indicates that Syk phosphorylation is induced by cross-linking DR but not FcγR. These differences in phosphorylation patterns were not due to the enhanced recovery of these kinases, as evidenced by the presence of an equal amount of Syk protein molecule in each sample (Fig. 8A and 8B). These data are consistent with results obtained using the Syk inhibitor piceatannol on IgM production, thereby collectively indicating that HLA-DR molecules on B cells not only present antigenic peptides to T cells, but also up-regulate IgM production, in association with Syk activation and without the involvement of Src kinases.

Kinetics of proliferation induced by cross-linking HLA-DR molecules on CD4+ T cells

We investigated kinetics of T-cell proliferation when HLA-DR molecules on CD4+ T cell were cross-linked by solid-phase mAbs (Fig. 9). Cross-linking by anti-HLA-DR mAb L243 stimulated T cells to proliferate while control Ab did not do so. The proliferative response reached maximum at 24 hr, as did the response induced by anti-CD3 mAb. T cells incubated with biotinylated anti-DR + avidin proliferated as well (data not shown). However, T cells stimulated by biotinylated anti-DR alone did not do so (data not shown). Thus not only binding of anti-DR mAb molecules but also their cross-linking is necessary for T cells to proliferate (25).

T cell clonal anergy induced by a soluble form of antigenic peptide, anti-CD3 mAb and anti-HLA-DR mAb

SF36.16 cells cultured with the soluble form of antigenic peptide for 7 days resulted in low responsiveness to irradiated PBMC in the presence of the indicated concentration of peptide (Fig. 10A). Thus, when these cells are re-stimulated with 1 nM antigenic peptide and irradiated autologous PBMC, there maybe only marginal proliferation, whereas SF36.16 cells cultured for 7 days with irradiated autologous PBMC prepulsed with 5 μM peptide did exhibit marked proliferative responses when re-stimulated with 1 nM peptide and irradiated

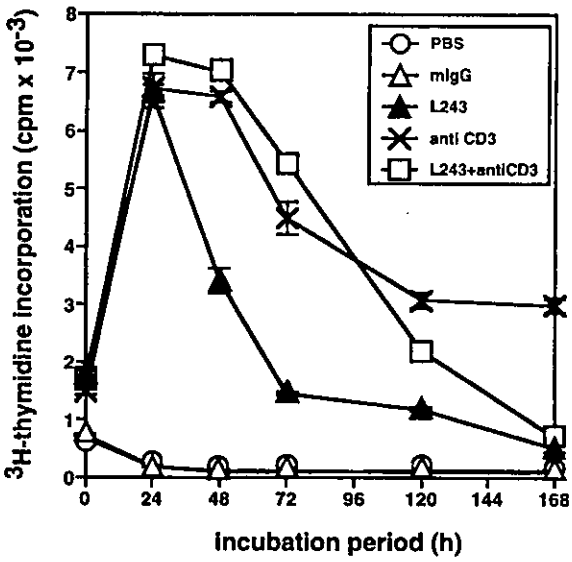

Figure 9. Kinetics of proliferation induced by cross-linking HLA-DR molecules on CD4$^+$ T cells. Kinetics of T-cell proliferation was investigated when HLA-DR or CD3 molecules on CD4$^+$ T cell were cross-linked by solid-phase mAbs (open circle, PBS; open triangle, control mIgG; closed triangle, anti-HLA-DR mAb L243; cross, anti-CD3 mAb OKT3; open square, L243 + OKT3). After indicated incubation periods (0-168 hr), T cells were cultured in the presence of 1 μCi/well [^{3}H]thymidine during the final 16-h period, and the incorporated radioactivity was measured by scintillation counting. All data are expressed as the mean value of duplicate determinations ± standard error. The experiment shown is representative of two independent experiments.

autologous PBMC (Fig. 10A).

SF36.16 cells cultured in the presence of solid-phase anti-CD3 mAb OKT3 for 7 days also showed low responsiveness (Fig. 10B). Interestingly, SF36.16 cells cultured in the presence of solid-phase anti-HLA-DR mAb L243 for 7 days also showed a low response (Fig. 10C). Furthermore, when exogenous IL-2 at 200 U/ml was added to re-stimulation the culture, the responsiveness was restored (p = 0.0041), although human recombinant IL-2 only marginally exhibited an enhancing effect in control cultures (p = 0.25; Fig. 10D). All these observations indicate that L243-stimulated T cells were rendered anergic. It is unlikely that solid-phase L243 blocked antigen presentation to T cells in the re-stimulation culture, because levels of responsiveness were the same, between L243-stimulated and control cultures, in the presence of IL-2 (p = 0.11; Fig. 10D).

Expression of CDK inhibitors p21^{Cip1} and p27^{Kip1} in anergic CD4+ T cells

As shown in Fig. 11, T cells with clonal anergy induced by stimulation with the soluble form of an antigenic peptide, expressed higher level of CDK inhibitor p27^{Kip1} on day7, than did those cultured with irradiated autologous PBMC prepulsed with 5 μM agonistic peptide. Likewise, anergic T cells induced by cross-linking its CD3 or HLA-DR, expressed higher level of CDK inhibitor p27^{Kip1} . In contrast, both expressed the same levels of CDK inhibitor p21^{Cip1} (Fig. 11), p15^{INK4b} and p16^{INK4a} (data not shown). Neither T cells stimulated with control IgG (Fig. 9) nor those stimulated with peptide-unpulsed PBMC showed proliferative responses. Therefore, T cells under such stimuli died on day 7, which did not enable us to test protein expression.

Cytokine production from fibroblasts by stimulus via HLA-II molecules

Since we did not observe proliferative responses of T cells when fibroblasts were used as APC, we then considered the possibility that HLA-DR molecules on fibroblasts may act as receptor molecules (27). The cytokine production from PDL was first evaluated by determining cytokine levels in culture supernatants, when fibroblasts were stimulated via HLA-DR molecules by anti-HLA-DR mAbs. The isotype-matched immunogloblin was used as a control. As shown in Figure 12, stimulated PDL produced larger amounts of IL-8, IL-6, MCP-1 and RANTES compared with control cells.

We then examined whether cytokine production from fibroblasts actually occurred when HLA-DR molecules on fibroblasts are ligated by their natural ligands. To address this issue, peptide pulsed fibroblasts were co-cultured with emetine-treated T cells. Since

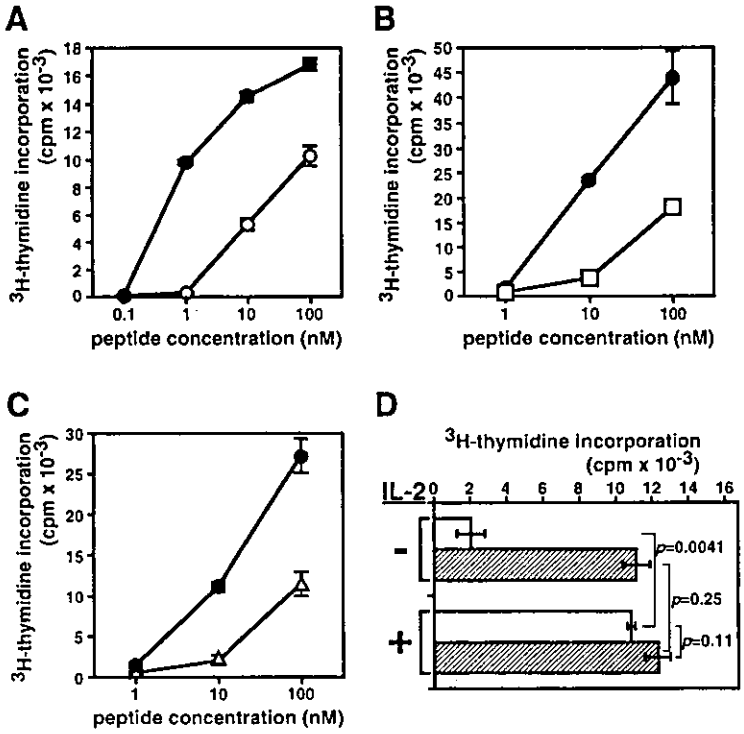

Figure 10. T cell clonal anergy induced by a soluble form of the antigenic peptide (A), anti-CD3 mAb (B) and anti-HLA-DR mAb (C, D). SF36.16 T cells were primarily stimulated with irradiated autologous PBMC prepulsed with the wild-type peptide (5 μM BCGap84-100 for 5 h at 37 °C; closed circle or hatched bar), soluble wild-type peptide (1 μM BCGap84-100; open circle), plastic immobilized anti-CD3 (OKT3; open square) or plastic immobilized anti-DR (L243; open triangle or open bar). Seven days after initiation of culture, T cells in these cultures were re-stimulated (secondary stimulation) with various concentrations of wild-type BCGap84-100 plus irradiated PBMC (A, B, C), or with 10 nM wild-type BCGap84-100 plus irradiated PBMC, in the presence or absence of 200 U/ml of exogeneous human recombinant IL-2 (D). The T cells were cultured on a 96-well plate for 72 h, and subjected to [^{3}H]thymidine incorporation assay. All data are expressed as the mean value of duplicate determinations ± standard error. Data are representative of two independent experiments. (D) Proliferative response of T cells to exogeneous IL-2. Primary stimulation, secondary stimulation and [^{3}H]thymidine incorporation assay were done as described above. Results are expressed as the means ± SD of duplicate measurement. Statistical significance was analyzed using Student's *t* test.

emetine is a *de novo* protein synthesis inhibitor, we first treated T cells with emetine to prevent T cells from up-regulating cytokines or cell-surface molecules. As shown in Figure 13, IL-8, IL-6, MCP-1 and RANTES were indeed produced from peptide-pulsed PDL following 16 hr culture ($1x10^4$ cells/well) with emetine-treated T cells, whereas no cytokines were produced when the cells were merely pulsed with peptide. Peptide-pulsed and IFN-γ treated PDL produced larger amounts of cytokines including IL-8, IL-6, MCP-1 and RANTES

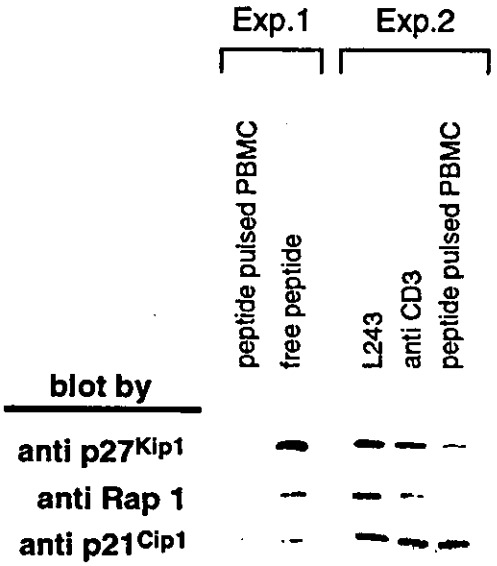

Figure 11. Expression of CDK inhibitors. T cell clone SF36. 16 were stimulated using a soluble form of antigenic peptide (1μM BCGap84-100), immobilized anti-CD3 (OKT3), immobilized anti-DR (L243) or irradiated autologous PBMC prepulsed with the wild-type peptide (5 μM BCGap84-100 for 5 h at 37 °C) at 37 °C. After 7 days of incubation, the T cells were lysed. Cell lysates were directly resolved on 13.5 % SDS-PAGE, transferred to nitrocellulose membranes then blotted using Abs to CDK inhibitor $p27^{Kip1}$ and CDK inhibitor $p21^{Cip1}$.

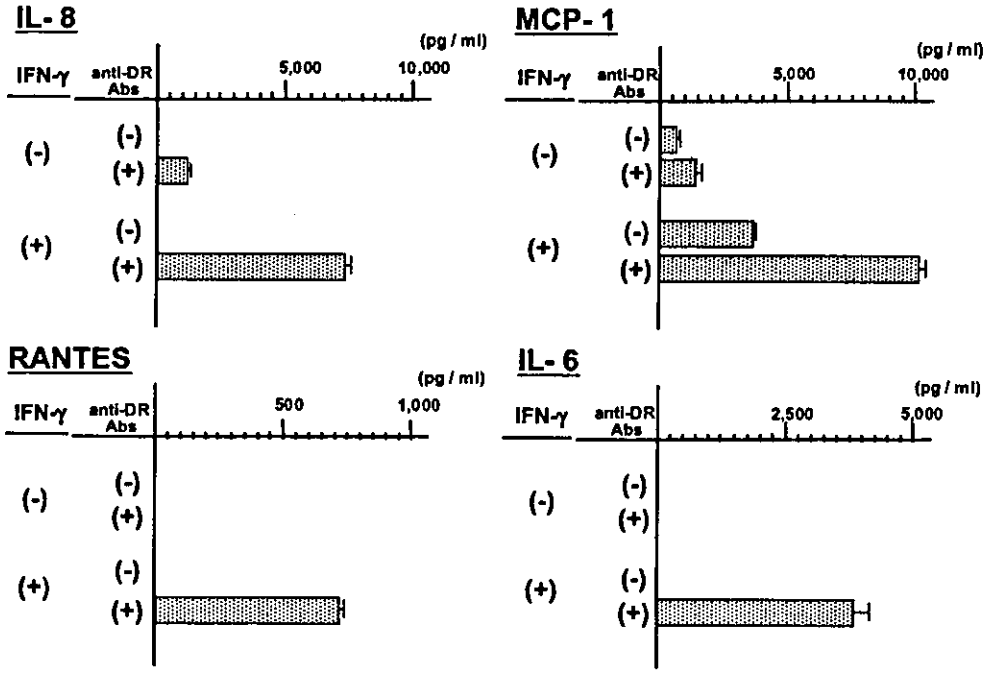

Figure 12. Cytokine productivity of PDL by the stimulus with anti-HLA-DR (L243) mAb. IFN-γ-treated or –untreated PDL ($1x10^4$ cells/well) were cultured with or without L243 in the presence of goat anti-mouse IgG2a Ab. The supernatants were collected following 16 hr culture of the cells, and concentrations of IL-1β, IL-6, IL-8, MCP-1 and RANTES were determined using ELISA kits as in "Materials and Methods".

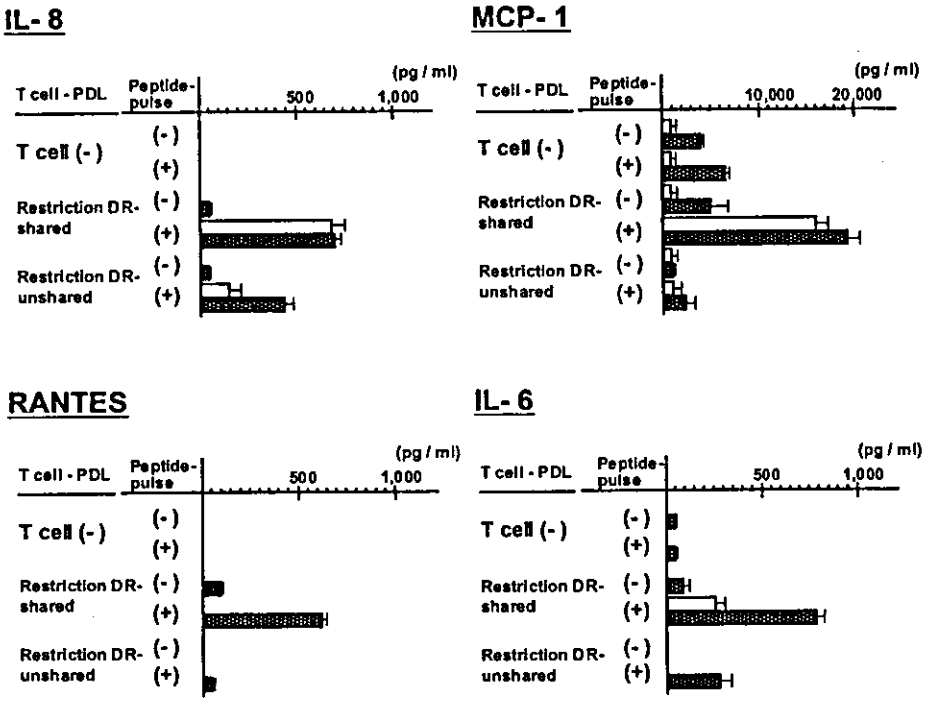

Figure 13. Cytokine productivity of PDL by stimulation via HLA-II molecules when PDL were co-cultured with emetine-treated T cells. PDL ($1x10^4$ cells/well) was pulsed with or without antigenic peptide in the serum free medium for 24 hr following 72 hr culture with or without IFN-γ before the cells were cultured with emetine-treated T cells. The supernatants were collected following 16 hr culture of the cells, and cytokine concentrations were determined. Shaded and open bars indicate the results of IFN-γ-treated and –untreated PDL, respectively.

compared with non-treated and non-pulsed PDL. Moreover, PDL expressing HLA-DRB1*1501 molecules produced the large amounts of IL-8, IL-6, MCP-1 and RANTES when emetine-treated T cells (restricted by DRB1*1501) was co-cultured with PDL. MCP-1 and RANTES were produced only when peptide-pulsed PDL was cultured with restriction DR-shared T cells, whereas IL-6 and IL-8 were produced even when restriction DR-unshared T cells were co-cultured. Although RANTES and IL-6 productivity was relatively higher in IFN-γ treated, peptide-pulsed PDL cultured with DR-restricted T cells, no significant difference was found in IL-8 and MCP-1 productivity between them.

DISCUSSION

The observation that IFN-γ / IL-4 produced by T cells are associated with HLA restriction molecules even in freshly isolated short-term T-cell clonal responses to crude protein antigens or randomized peptide antigens, is evidence that the phenomenon is not limited to three T-cell clones used in this study. Thus, although not being complete as shown in Fig. 3, HLA class II subregions may determine T cell differentiation patterns or IFN-γ / IL-4, probably through monocyte responses. However, one might speculate that DR-peptide complex deliver strongest avidity between TCR, leading to Th1-prone responses (28,29). However, absence of DR-restricted T cell clones

with low IFN-γ / IL-4 rules out this possibility, because low-affinity DR-binding peptides should exist, which should activate Th2-prone responses, if the phenomenon is attributed to avidity alone. Indeed, it is likely that such a phenomenon is attributed to high IL-12 production through DR signaling (Table 1).

PKC and Syk are associated (30) and involved in signaling through MHC molecules. We observed a slight enhancement of monokine production by PKC inhibitor GF109203X and Syk inhibitor piceatannol. The precise mechanisms are yet to be determined, but one might speculate that they negatively regulate monokine production induced by ligating class II HLA.

Because transmembrane and intracellular domains are markedly different among α and β chains of HLA-DR, -DQ and -DP, and MHC molecules have no immunoreceptor tyrosine-based activation motif, it is reasonable to speculate that HLA-DR, -DQ or -DP molecules are associated with distinct signal transduction molecules. Differential endosomal trafficking / recycling (31), differential signaling in monocyte subsets (32), including contaminated dendritic cells, and even differential localization in membrane microdomains would also need to be considered. Study is currently underway, to address these questions, using various monocytic cell lines and mass mapping techniques.

IgM contributes to early defense against microbial infections (33). When B cells are exposed to non-self antigens, such as those of microbial origin, B cells bearing surface IgM specific for the antigen are capable of concentrating the antigen and present it effectively to T cells. We found that cross-linking DR molecules up-regulates not only secretory-type but also membrane-type μ chains, which may indicate that cross-linking DR molecules leads to more effective antigen presentation. It is also important to note that CD40-generated signals arrest B-cell terminal differentiation to produce Igs (34). Although DR-mediated signals appear to up-regulate IgM production in the absence of CD40-CD154 interaction, further investigation is needed to determine whether or not the generation of signals via CD40 under physiological T-B interactions interferes with IgM production induced by DR-mediated signals. In this study, ligation of DR molecules not only with specific Abs (either solid-phase Abs or soluble Abs), but also with HLA-peptide-TCR interaction, induced IgM production, suggesting that signals via DR alone are capable of inducing up-regulation of IgM, which may also occur in physiological T-B interactions. In this relation, DR-mismatched transplantation should be one of rare cases, in which massive T-B interaction via DR occurs *in vivo*. Indeed, when DNA typing of HLA-DR was unavailable, graft-vs-host disease was frequent, and such patients reportedly had deposition of IgM at the dermo-epidermal junction. (35).

BCR-Ag-complex is internalized to supply T-cell epitopes, and subsequent DR-peptide-TCR interaction results in class switching, which eventually leads to decreased IgM production (36). Indeed, our experimental system did not allow BCR to interact with protein antigens, and T cells were treated with emetine (thereby bearing no class switch pressure). Such a system might have up-regulated IgM to be readily detected. However, because the disappearance of surface IgM at antigen presentation (before class switching) is incomplete, one might speculate that signaling through DR supplies new IgM molecules, for a short and critical period of time for T-B interaction before class switching is initiated. Other factors should also be considered, because even with thymus-independent antigens, IgM production from B cells can be induced (37).

In case of the anergy induced by soluble antigenic peptide, signals should be transmitted to T cells not only via TCR but also via class II HLA because T cells express both TCR and HLA-DR. Although anergy induced by the lack of co-stimulation was rescued by signaling via CD28, anergy induced by soluble antigenic peptide was not rescued (38), which is also the case in our present study (data not shown). It is therefore likely that molecular mechanisms differ between anergy induced by the lack of co-stimulation and that induced by anti-DR, even though the behavior of CDK inhibitors are apparently the same.

Fibroblasts are known to participate in the immune system because of their expression of several immunoregulatory molecules on their cell surfaces, as well as many cytokine species. It was reported that fibroblasts produce IL-8 by the stimulus with several inflammatory cytokines (IL-1β and TNF-α; 39,40) and LPS (41,42). A previous study demonstrated that the engagement of MHC class II molecules by the superantigen including SEA and *Mycoplasma arthritidis*-derived superantigen induced the RANTES, MCP-1 and IL-8 mRNA expression in synovial fibroblasts (43). Herein, we demonstrated that PDL$^-$ produced large amounts of chemokines when cultured with emetine-treated T cells, under restriction DR-shared conditions. Thus, signaling via HLA-II molecules into fibroblasts is not only induced by the engagement of MHC class II molecules with superantigens, but also by making the DR-peptide-TCR complex.

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抗原特異的免疫療法

Antigen-specific immunotherapy for allergy

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◎小児期における減感作療法の成功は，アレルギーマーチ(さまざまな抗原に対して鼻炎，喘息などさまざまな症状が加齢とともに現れてくること)の進行を有意に抑えることが知られている．アレルゲンを用いた減感作の標的はT細胞であるため，抗原をさまざまな形に修飾/選択したりすることにより，その効果を確実にすることができる．減感作におけるアナフィラキシーの副作用はアレルゲンがB細胞エピトープを複数有することによるが，この点においてもT細胞減感作は有利である．抗原そのものの構造以外にも抗原提示分子の選択や，粗アレルゲンからのTh2アジュバント活性の除去も考慮されるようになるであろう．

Key word　アレルゲン，アレルギー，ペプチド，減感作，アジュバント

抗原特異的な治療の原点はアレルゲン蛋白分子を用いた減感作療法にある．その作用機序についてはいまだに議論が絶えないが，IgGクラス(IgG4主体)のブロッキング抗体を誘導する細胞成分はIL-10産生性のregulatory T細胞で完全に説明されるかもしれない．蛋白分子そのものを抗原として用いる場合に比べて，その断片であるペプチドを用いる場合は，①合成が容易である，②アナフィラキシーなどの副作用が起こりにくい，③したがって一度に大量を投与できる，④抗原の構造を治療に有利なように変換できる，などの利点がある．

本稿ではこれらの基本的考え方について，実例を示しながら概説したい．

T細胞エピトープをそのまま用いる場合

外来蛋白(アレルゲン)は抗原提示細胞によりプロセシングされ，ペプチド断片としてMHC class II分子の抗原収容溝内に結合する[1]．この複合体をT細胞抗原レセプター(TCR)によって認識したT細胞が活性化されて抗原特異的免疫応答がはじまる．

ここで，T細胞エピトープとはT細胞によって認識される抗原の一部分であり，B細胞エピトープとはB細胞によって認識される，すなわち抗体と結合する抗原の一部分である．B細胞エピトープは可溶性抗体と直接結合するため，球状蛋白の場合，水(極性溶媒)と接する蛋白表面に存在することになる．これは，生化学的にいえば，親水性残基の占める割合が多い部分にあたる．加えて抗体は抗原側の単純な一次構造のみならず，高次構造をとったときにのみ形成されるようなコンパクトな構造，糖鎖，リン酸化部分などをも認識できることが特徴である．それに対して，T細胞エピトープ(MHCとともにTCRによって認識されるペプチド部分)は断片化されてMHCの抗原ペプチド収容溝に入るわけであるから，かならずしも水分子と接する部分に存在する必要はない(図1)．生体が蛋白Xで免疫されて抗X抗体を産生するようになる場合，まずXに特異的なヘルパーT細胞が誘導されて続いて抗X抗体を産生するB細胞が活性化されるわけであるが，T細胞がMHCとともに認識するX上のアミノ酸配列は，抗体が結合するX上のアミノ酸配列とは多くの場合異なっている．

したがって，アレルゲン上のT細胞エピトープ

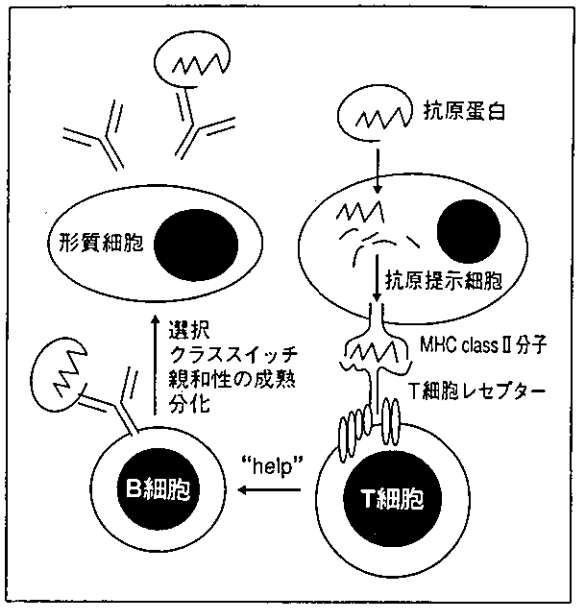

図 1　T 細胞エピトープと B 細胞エピトープ
MHC とともに T 細胞抗原受容体によって認識される断片化された抗原ペプチドを T 細胞エピトープとよぶ．これに対して B 細胞エピトープは，直接抗体と結合できる．抗体と結合できる B 細胞は，ヘルパー T 細胞の存在下に増殖する過程でクラススイッチ，親和性の成熟などを経て，形質細胞へと分化する．抗原特異的な B 細胞が増えると，抗体を介して抗原を B 細胞内に取り入れて分解することにより，抗原提示の効率はさらによくなる．各種蛋白分子の大きさは誇張して描かれているが，実際の大きさは細胞の千～数千分の 1 である．MHC 分子や T 細胞抗原受容体は 1 細胞表面当り 1 万～10 万分子発現している．

を含むペプチド断片は，B 細胞エピトープを含まない，あるいは含んでいても一価であり，高親和性 Fcε レセプターをクロスリンクできないなどの理由により，患者に投与してもアナフィラキシーなどの副作用が起こらないことが多い(図 2)．

さらに興味深いことに，T 細胞エピトープを含むペプチドを生体に投与すると，T 細胞が抗原特異的に不活化(アネルギー)される現象が知られている[2]．

このような理論的背景をもとに，ネコの毛アレルゲン(*Fel d* I)のペプチドを用いた減感作の臨床治験がはじまった．そもそも欧米人を対象として T 細胞エピトープの同定が行われているため，日本人にそのまま適応できるかどうかに関しては結論を待ったほうがよい(HLA 型は人種差が大きいので，結合できるペプチドの種類も異なる[3]；図 3)．この方法の利点は，初期から大量に投与してもアナフィラキシーの可能性が少ないため思い切った短期のプロトコールを試すことができる点にある．逆にペプチドであるため，最大公約数的に主要 T 細胞エピトープをカバーするようにペプチドをデザインするしか手がないという難点もある[4]．

一残基置換ペプチドを用いる場合

抗原ペプチド上の，おもに TCR による認識にかかわるアミノ酸残基を置換したアナログペプチドと，MHC 分子との複合体に対する T 細胞クローンの応答を解析することにより，T 細胞の応答は all or none 型のものではなく，TCR のリガンドの構造の変化に対応して量的あるいは質的に変化することが明らかにされた[5]．ペプチドは一残基進むごとに 130 度左向きに回転する構造をとるため，残基によって MHC と相互作用するか，TCR と相互作用するかが異なる．一般的にいって，HLA と

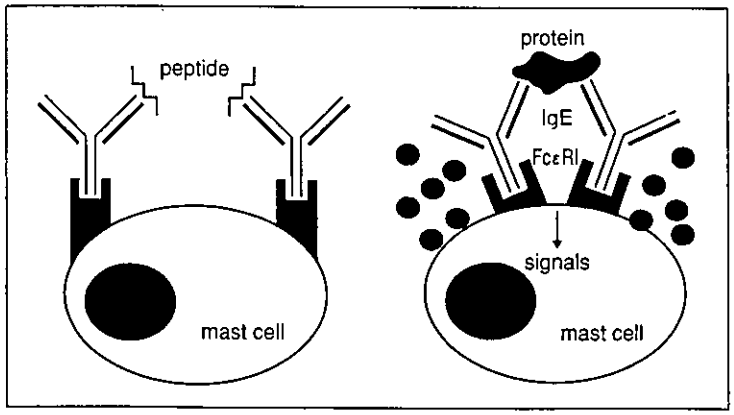

図 2　1 価のペプチドと多価の蛋白抗原の比較

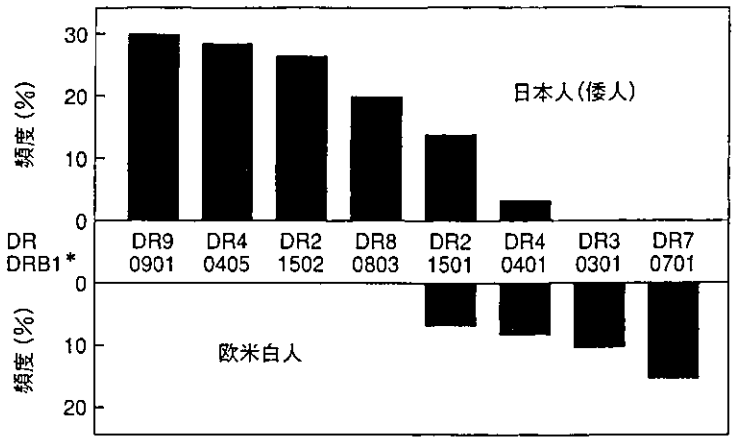

図 3 特定の HLA-DRB1 対立遺伝子の頻度
頻度は日本人と欧米白人とでは著しく異なる.

相互作用する残基を置換すると免疫応答の量的変化が，また，TCR と相互作用する残基を置換すると免疫応答の質的変化が誘導されやすい．質的変化の代表的なものは TCR アンタゴニズムであるが，アネルギーの誘導，サイトカインの産生変化，T 細胞の寿命の変化なども観察される[5]．

著者らの研究では，アナログペプチドが T 細胞応答を TCR 由来のシグナルで直接修飾する経路以外にも，HLA 分子を介したシグナルによってモノカインの産生に変化が誘導され，その結果として T 細胞応答が変化する例も観察されている[6]．実際，アレルギーや実験自己免疫疾患において，動物に抗原ペプチドのアナログを投与することにより疾病の発症を予防あるいは治療する方法が報告され，脚光を浴びている[7]．

多残基置換ペプチドを用いる場合

T 細胞応答を抗原特異的に増強する試みはおもに感染免疫や腫瘍免疫の領域でなされてきたが，自己免疫やアレルギーにおいても，Th1 応答へのシフトやクローン特異的なアポトーシスの誘導と関連して注目されはじめている．抗原構造の修飾に関しては一残基置換アナログペプチドによる免疫応答の修飾が数多く報告されているが，HLA との結合を増強する[5,8]にしても，TCR との結合を増強するにしても，log オーダーの増強は難しく，あらたな戦略の開発が望まれていた．

Hemmer らは combinatorial peptide library を用いた T 細胞エピトープの positional scanning により，野生型ペプチドよりも log オーダーで強い抗原刺激活性を有するペプチド(peptide superagonists)を同定した．これは一残基を置換したペプチドではなく，多残基を置換したペプチドであった[9]．その後，著者らを含めて複数のグループが，異なる T 細胞クローンを用いて同様の戦略を試みたが，“ペプチド上のあるポジションにおいて免疫応答増強的に働くアミノ酸残基置換をすべてのポジションに有するペプチドは，かならずしもスーパーアゴニストではない”ことが明白になってきた[5,10]．これはペプチドの構造を考えれば自明であって，特定のアルファ炭素の周囲の回転角は数残基先のアルファ炭素の周囲の回転角にも影響を与えるからである[11,12]．よって，ちょうどチェスのゲームにおいてコンピュータがとる思考過程のようにファジーな戦略をとらないと，“最強の”ペプチドを同定することは難しい．

紆余曲折の末，著者らは combinatorial peptide library に逆相 HPLC と質量分析を組み合わせることによりこの問題を解決した[10]．詳細は他著を参照されたい[10,13]．また，著者らは最近，このような方法を用いて，抗原特異性がわからない T 細胞クローンが認識するペプチドを同定する方法を報告した[14,15]．

ペプチドの長さを変えて用いる場合

以上紹介したスーパーアゴニストも含めて，こ

れまでのアナログペプチド免疫療法のアイデアは，特定のT細胞レパートリー(クローン)を標的にしたものであった．スーパーアゴニストの場合，そのクローンのみの応答を増強することによっても目的は達成されると考えられるが，病気の原因となっている免疫応答を抑制しようとする場合には特定のT細胞クローンを標的とするだけでは問題解決にならないことのほうがはるかに多い．別のいい方をすれば，多クローン性応答が標的となることが多い．

著者は，T細胞応答に及ぼすランダムな配列のペプチドの影響を観察してみた[16]．まず，すべてのポジションにCys以外のアミノ酸を含むX*n*ペプチド(*n*=9, 11, 13, 15, 17, 19)を合成した．これらのペプチドでPBMCを直接刺激してもほとんど増殖反応は誘導されず，逆にX13やX15では抑制される傾向があった．そこで，PPD，ダニ抗原，混合リンパ球培養反応(MLR)など，PBMCに多クローン性応答を誘導することがよく知られている免疫応答の系に対するX*n*ペプチドのTCRアンタゴニズム活性を検討した．面白いことに，中途半端な長さのペプチド(X13やX15で最大活性)において濃度依存性の抑制が観察された．この培養上清をPPDに対する増殖応答の系に添加してみても抑制活性はみられないことから，可溶性因子による抑制ではないことが判明した．また，DRB1*0405+peptide Xを認識するMLRをDRB1*0405の高親和性結合ペプチド(Kd=70nM)[3]で抑制しようとしても抑制されないことから，X15の効果はHLA-ペプチドの結合抑制ではないことが明らかである．これらの観察から，X15による抑制はTCRアンタゴニズムによるものであると推測された．

おそらく，多クローン性のT細胞応答のなかの成分であるさまざまな単クローン性応答にTCRアンタゴニストとして作用できるペプチドがX*n*のなかに含まれているからであろうと考えられる．実際，T細胞クローンを標的としてX13やX15を作用させても抑制が観察され，特異的なペプチドを用いた場合，C末端から短くした，しかも長めのペプチドは強力なTCRアンタゴニストであった．このようなペプチドは異なる範囲のペプチドを認識するT細胞クローンの増殖を抑制することはなく，N末端から短くしてもこのような現象はみられない．つまり，ペプチドが中途半端な長さであると，C末端側が短くかつN末端側が長すぎないようなペプチドを含む確率が高くなり，それによってTCRアンタゴニストの含有率が高くなるものと思われる．

ペプチドのN末端側の第一アンカーはMHCとの結合においてたいへん重要であるから，N末端から短くしたものはMHCとの結合そのものができなくなり，TCRアンタゴニストではありえない．それに対して，C末端から短くしたものは，P7, 8, 9の残基がなくなってもMHCとの結合性は維持しており[17]，その結果，短縮ペプチドがちょうど一残基置換によるTCRアンタゴニストと同じような形でT細胞から認識されるのであろう．このようなペプチドは，同じ抗原ペプチドを認識するにもかかわらずTCR usageが異なるようなT細胞レパートリーに共通のTCRアンタゴニストになりうることから，抗原特異的かつTCR非特異的な免疫抑制効果を有している．このような短いペプチドの生体内での効果はマウスのEAEモデルで確認されている[18]．

抗原提示分子を選択して用いる場合

HLA+ペプチドがTCRと相互作用を起こすとT細胞側にシグナルが入りT細胞が活性化されるのは，前述のようによく知られた事実である．一方，T細胞-抗原提示細胞相互作用により誘導される抗原提示細胞側へのシグナリングに関してはCD40などがよく知られているが，MHCそのものも重要な役割を担っているという報告は多い．1990年前後からMHC class II分子は単に抗原断片を提示するだけではなく，TCR-ペプチド/MHC複合体が相互作用を起こした際にMHC class II分子からシグナルが入り抗原提示細胞も活性化されることがわかってきた[19]．著者らは，固相化抗体またはエメチン処理したT細胞クローンと共培養することにより末梢血単球上に発現するDR, DQ, DP分子を架橋した[20]．MAPK/Erkの活性化はDRを介した刺激でのみ観察されたが，MAPK/p38の活性化はDR, DQ, DPすべてを介した刺激