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Research Article

Activated mouse T cells downregulate, process and present their surface TCR to cognate anti-idiotypic CD4$^+$ T cells

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Summary The ability of activated T cells to present foreign antigens through the MHC class II pathway has been shown in the case of human, rat and mouse T cells. In the present study, the ability of activated T cells to present their endogenous TCR in association with MHC class II molecules to CD4$^+$ T cells was shown. Upon activation mouse T cells downregulate their surface TCR, which are degraded into peptides in endosomal/lysosomal compartments. The idiopeptides (peptides derived from the variable region of the TCR) are presented to cognate anti-idiotypic CD4$^+$ T cells, resulting in activation and proliferation of these cells. Interaction of idiotypic and anti-idiotypic T cells brought about by presentation of TCR idiopeptide may have important implications for T-cell vaccination and perpetuation of T-cell memory not requiring persisting antigen or long-lived memory cells.

Key words: antigen presentation, anti-idiotypic T cell, T-cell memory, T-cell receptor (TCR).

Introduction

T-cell immune responses are initiated by TCR recognition of peptide–MHC (pMHC) on APC.[1] After specific interaction of T cells with APC, TCR and MHC molecules are assembled at the centre of a supramolecular activation cluster.[2–4] TCR–CD3 complexes on the T-lymphocyte surface are rapidly downregulated upon activation with pMHC complex, superantigen or cross-linking with anti-TCR or anti-CD3 antibodies.[5–7] Receptor downregulation is a common phenomenon shared by other membrane receptors with intrinsic or associated tyrosine kinase activity.[8] This downregulation of TCR may help in terminating cellular responses by reducing the number of receptors at the cell surface or by uncoupling receptors from membrane-anchored signalling molecules.[9] This effect is supported by the observation that TCR–CD3 downregulation results in a loss of cellular sensitivity to subsequent stimulation.[10–12] It is also believed that TCR–CD3 internalization plays an important role during T-cell activation by allowing serial triggering of multiple TCR–CD3 complexes by a few antigen–MHC complexes.[13] The TCR and associated signalling molecules are recycled back to the cell surface, and TCR molecules are also partially degraded in the endosomal compartments. This activation-driven downregulation and degradation of TCR molecules probably form a part of the peptide pool for the MHC class II molecules. Activated T cells are known to synthesize and present antigen through MHC class II molecules.[14–17] The presentation of idiopeptide of TCR (peptide derived from the variable region of the TCR) by activated T cells may lead to triggering of cognate anti-idiotypic T cells. Anti-idiotypic T cells have been proposed to control the self-reactive T-cell population in autoimmune diseases after T-cell vaccination,[18,19] and have been hypothesized to play a role in perpetuation of T-cell memory.[20,21] The pathway for generation of anti-idiotypic T cells is not well defined. Presentation of idiopeptides by activated T cells may lead to T cell–T cell interaction, which is a proposed mechanism for T-cell vaccination, control of autoimmune disease and maintenance of T-cell memory.[18–21]

In the present study, we show that activated mouse T cells downregulate their surface TCR. The surface TCR are degraded by lysosomal proteases and presented in the context of MHC class II molecules. The presentation of TCR peptide through MHC class II molecules by activated T cells (idiotypic T cells) leads to its interaction with cognate anti-idiotypic CD4$^+$ T cells. The interaction of idiotypic T cells with anti-idiotypic CD4$^+$ T cells leads to proliferation of anti-idiotypic T cells as well as cytokine secretion in an MHC class II-dependent manner.

Materials and methods

Animals

BALB/c mice were bred and maintained in the Central Animal Facility, Indian Institute of Science, Bangalore, India. Six- to eight-week-old male mice were used in all experiments. All of the experiments were carried out following institutional animal ethical committee guidelines.

Reagents

Purified antimouse I-A/I-E (clone 2G9), antimouse CD11c (clone HL3) and isotype control antibodies were purchased from Pharmingen (San Diego, CA, USA). Polyclonal rabbit antimouse Ig, antimouse TCRα/β-FITC (clone H57-597) and antirat tetramethyl rhodamine isothiocynate (TRITC)-conjugated antibody were purchased from Sigma (St Louis, MO, USA). Antirat IgG-FITC was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Anti-lysosomal-associated membrane protein (LAMP)-1; 1D4B antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antimouse

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TCRβ–PECy5 (H57-597) and streptavidin–FITC were purchased from e-Bioscience (San Diego, CA, USA). Rinderpest virus nucleocapsid protein (N protein) and phosphoprotein (P protein) were expressed in *Escherichia coli* and purified, as described earlier.[22,23]

Purification of T cells

T cells were purified as described earlier.[24] Briefly, mice were killed by cervical dislocation. Inguinal, posterior axillary, internal axillary and popliteal lymph nodes were removed, and a single cell suspension was made. Cells were panned for 1 h on a fetal bovine serum (FBS; Gibco Invitrogen, Carlsbad, CA, USA) coated plate (Falcon). Unbound cells were harvested, and complement-mediated lysis was carried out using antimouse Ig, antimouse MHC class II (clone m5/115), antimouse CD11c and rabbit complement. Mononuclear cells were purified by density gradient centrifugation on Histopaque-1077 (Sigma). Cells were further panned on antimouse Ig antibody coated plate for 1 h in complete medium (RPMI-1640 supplemented with 25 mmol/L HEPES, 100 μg/mL penicillin, 100 μg/mL streptomycin, 20 μg/mL gentamicin and 10% FBS). Unbound cells were harvested, and purity of T cells was checked by FACS and found to be 99%.

In vitro *activation of T cells*

Purified T cells (4×10^6) were activated with PMA (500 ng/mL) and ionomycin (50 ng/mL) for 12 h in complete medium in a final volume of 1.5 mL in each 24-well plate. Cells were harvested and washed with HBSS. Activation of T cells was monitored by surface staining for CD69 and analysed by FACS. The viability of T cells before and after activation was detected by trypan blue dye exclusion test and was found to be 98%.

FACS analysis

Purified T cells were stained with a saturated amount of purified antimouse TCRβ−PECy5 or isotype control antibody. Briefly, cells were incubated in PBS containing 10% FBS for 20 min on ice. Cells were washed with PBS containing 0.2% BSA and 0.01% sodium azide and then they were incubated with fluorochrome-conjugated antibody for 30 min on ice. Cells were washed with PBS. Flow cytometric analysis was carried out on FACSscan (BD Bioscience, San Jose, CA, USA), and the data were analysed using WinMDI 2.8 software (http://facs.scripps.edu).

Generation of idiotypic T cells

Mice were s.c. immunized with N protein (50 μg) in CFA, and boosted after 3 weeks with N protein (50 μg) in IFA. One week after the booster was administered, draining lymph node cells were harvested. T cells from these mice were purified as described above. The antigen specificity of T cells was ascertained by the ability of these cells to proliferate *in vitro* in response to N protein.

Generation of anti-idiotypic T cells

Purified irradiated idiotypic T cells (20×10^6 cells) were given intraperitoneally to syngenic BALB/c mice. After 3 weeks, mice were boosted with the same number of irradiated idiotypic T cells. Lymph node T cells from immunized mice were purified as described above and used as anti-idiotypic T cells.

Downregulation of TCR

Purified naive mouse T cells were stimulated with PMA/ionomycin in complete medium in the presence or absence of cytochalasin D (20 mmol/L; Sigma) for different time periods. Cells were fixed with 2% paraformaldehyde. Cells were incubated in PBS containing 10% FBS for 20 min on ice. Cells were washed with PBS containing 0.2% BSA and 0.01% sodium azide and stained with a saturating concentration of anti-TCRα/β-FITC antibody for 30 min on ice. Cells were washed and then analysed by FACS. Relative mean fluorescence intensity (MFI) was calculated by dividing the MFI of experimental value with MFI of isotype control antibody.

Confocal microscopy

Purified naive T cells were *in vitro* activated with PMA/ionomycin for 3 h in the presence or absence of chloroquine (40 mmol/L) and cycloheximide (40 μmol/L) in complete medium. Cells were washed with PBS, and intracellular staining was carried out for LAMP-1 and TCRα/β. Briefly, cells were blocked with PBS containing 10% FBS for 10 min on ice followed by incubation with purified antimouse TCRα/β antibody for 30 min on ice to block the surface TCR. Cells were washed and fixed with 4% paraformaldehyde for 15 min on ice. Cells were washed and permeabilized with permeabilizing solution (e-Bioscience) for 10 min on ice followed by incubation with purified anti-LAMP-1 antibody as primary antibody in the presence of permeabilizing solution containing 0.3% BSA for 30 min on ice. Cell were washed and stained with anti-TCRα/β-FITC (H57-597) and antirat-TRITC-conjugated antibody for 30 min on ice. Cells were washed twice with permeabilizing solution and once with PBS. Cells were visualized under a confocal microscope with a ×100 objective (Leica Microsystems, Wetzlar, Germany).

Purification of $CD4^+$ T cells

$CD4^+$ T cells were purified from the T-cell population by complement-mediated lysis of $CD8^+$ T cells as described earlier.[25] Purified T cells were incubated with antimouse CD8 antibody (clone 3.155) on ice for 30 min. Cells were washed and then treated with rabbit complement for 20 min on ice, and dead cells were removed by Histopaque-1077 density gradient centrifugation. Depletion of $CD8^+$ T cells was tested by FACS using antimouse CD4-FITC (clone H129.19) and antimouse CD8-FITC (clone 53-6.7), and purity of $CD4^+$ T cells was 99%.

Cell adhesion assay

Purified idiotypic T cells were treated *in vitro* with PMA/ionomycin for 12 h in the presence or absence of chloroquine (40 mmol/L) in complete medium. Cells were fixed with 0.2% paraformaldehyde. Anti-idiotypic $CD4^+$ T cells were harvested, washed and stained with carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene, OR, USA) as described earlier.[26] Briefly, purified anti-idiotypic $CD4^+$ T cells (5×10^6 cells) were suspended in 1 mL of PBS (pH 7.4). CFSE was added to a final concentration of 5 μmol/L and incubated for 10 min at 37°C by mixing twice in between. Cells were washed with PBS containing 20% FBS followed by complete medium. CFSE-labelled anti-idiotypic $CD4^+$ T cells (1×10^6 cells) were incubated with unlabelled idiotypic T cells (5×10^6 cells) or control T cells in a final volume of 200 μL complete medium for 30 min with intermittent shaking at 37°C. Cells were disturbed by tapping and then transferred to a glass slide and visualized under a fluorescence microscope (Leica). The total number of clumps present in a field with more than three cells and the number of clumps with CFSE-labelled cells were counted. More than eight random fields were examined. Adhesion efficiency were calculated as

$$\text{Adhesion efficiency (\%)} = \frac{\sum \text{Number of clumps present with CFSE-labelled cells}}{\sum \text{Number of clumps with or without CFSE-labelled cells}} \times 100$$

Surface biotinylation

Surface biotinylation was carried out as described earlier.[7] Briefly, cells were washed twice in PBS and resuspended in a freshly prepared solution of 0.5 mg/mL *N*-hydroxysuccinimido biotin (NHS-biotin)/PBS (Sigma). One millilitre of NHS-biotin solution was used per 16×10^6 cells. The cells were incubated on ice for 30 min and gently mixed every fifth minute. Subsequently, the cells were washed twice in PBS and resuspended in RPMI medium containing 20% FBS to a concentration of 1×10^6 cells/mL for degradation study. The biotinylation was monitored by FACS and fluorescence microscopy after staining with streptavidin–FITC (e-Bioscience).

Immunoprecipitation and western blotting

Biotinylated T cells (4×10^6 cells/sample) were stimulated with PMA/ionomycin for different time periods (0, 2, 4, 6, 8 h) in the presence or absence of chloroquine (40 mmol/L) and lysed in lysis buffer (50 mmol/L Tris, pH 7.5; 150 mmol/L NaCl; 1 mmol/L $MgCl_2$; 5 mmol/L EDTA; 1% NP-40 and protease inhibitor cocktail [Pharmingen]). Lysates were precleared with protein A-agarose beads (Bangalore Genei, Bangalore, India) and immunoprecipitated with anti-TCRβ antibody (H57.597) and protein G-sepharose bead (Sigma). The beads were washed four times with washing buffer (50 mmol/L Tris-HCl, pH 7.5; 150 mmol/L NaCl, 1 mmol/L $MgCl_2$ and 0.5% NP-40) and resuspended in incubation buffer (50 mmol/L Tris-HCl, pH 6.8, 2% SDS, 10% glycerol and 0.01% bromophenol blue) and boiled for 5 min and electrophoresed on 12% SDS–PAGE gels. The separated proteins were transferred to a nitrocellulose membrane and immunostained using horseradish peroxidase (HRP)-conjugated streptavidin (Bangalore Genei, Bangalore, India) and visualized with ECL Plus (Amersham Pharmacia Biotech, Little Chalfont, UK).

Idiopeptide presentation by T cells

To study the presentation of idiopeptide (peptide derived from the hypervariable region of TCR) by T cells to other cognate reactive T cells, antigen-specific T cells (idiotypic T cells) were generated by priming the mouse with N protein in CFA. After 5 days, draining lymph node cells were harvested, and a single cell suspension was made. T cells were purified and *in vitro* activated with PMA/ionomycin for 10 h. After incubation, cells were washed and fixed with 0.5% paraformaldehyde and used as APC.

Anti-idiotypic $CD4^+$ T cells were generated by adoptive transfer of irradiated idiotypic T cells (20×10^6 cells) intraperitoneally into syngenic mouse and boosted after 3 weeks with the same number of idiotypic T cells. Lymph node cells were harvested after 1 week of booster. This protocol efficiently generates anti-idiotypic T cells.[21] $CD4^+$ T cells were purified by complement-mediated lysis as described above.

To test the presentation of idiopeptides by idiotypic cells to their cognate anti-idiotypic $CD4^+$ T cells, anti-idiotypic $CD4^+$ T cells (4×10^5 cells/well) were cultured with paraformaldehyde-fixed idiotypic cells (1×10^5 cells/well) in complete medium. Cells were incubated for 48 h, and supernatants were harvested. Secretion of IL-2 was measured by ELISA as per the manufacturer's instructions (e-Bioscience).

In vitro *proliferation assay*

Purified idiotypic T cells were activated with PMA/ionomycin for 12 h. Cells were fixed with 0.2% paraformaldehyde and used as APC. Anti-idiotypic $CD4^+$ T cells were cultured in the presence of a fixed number of idiotypic cells (4×10^5 cells/well) in a final volume of 200 μL complete RPMI medium in U bottom 96-well plates. Plates were incubated for 72 h at 37°C in a 5% CO_2 incubator. Cells were pulsed with [^{3}H]thymidine (0.037 MBq/well; 74 GBq/mmol, PerkinElmer, Boston, MA, USA) in the last 16 h of incubation. Cells were harvested on glass fibre filters using a semiautomated cell harvester (Nunc, Roskilde, Denmark). Incorporated radioactivity was monitored using a Beckman scintillation counter.

Results

Downregulation of TCR by activated T cells

To monitor the downregulation of surface TCR by activated mouse T cells, purified T cells from naive mice were *in vitro* stimulated with PMA/ionomycin, and the surface expression of TCR was monitored. The results show that after activation, surface TCR are downregulated (Fig. 1a). To detect the role of actin molecules in the internalization of surface TCR, cells were treated with cytochalasin D during activation. Cytochalasin D inhibits actin polymerization, thereby inhibiting the inward movement of surface molecules.[27] The results show that cytochalasin D inhibits internalization of TCR, indicating that actin polymerization plays a role in downregulation of TCR.

To measure the kinetics of TCR internalization, T cells were stimulated for different time points and stained with a saturating amount of FITC-conjugated anti-TCRα/β antibody. The result shows that internalization increases as a function of time (Fig. 1b).

Localization of TCR and LAMP-1

To test whether internalized TCR goes to lysosomal compartment, colocalization of LAMP-1 and TCR was monitored by confocal microscopy. LAMP-1 is a late endosomal and lysosomal marker.[28] The result given in Figure 2 shows that internalized TCR goes to endosomes/lysosomes for its degradation, as shown by colocalization of LAMP-1 and TCR molecules. When cells were treated with chloroquine, which inhibits lysosomal enzyme activity, TCR accumulates in the lysosome/endosome. To rule out the possibility of newly synthesized TCR contributing to the lysosomal degradation, cells were treated with cycloheximide before activation. The results show that the colocalization is contributed by preformed TCR molecules, that is, TCR from surface of the T cells and not the newly synthesized TCR.

Degradation of internalized TCR

A schematic representation of detection of TCR degradation is shown in Figure 3a. To show that internalized surface TCR is degraded by lysosomal enzymes, the T cells were biotinylated at the cell surface and *in vitro* activated with PMA/ionomycin. The biotinylation was detected by FACS after staining with streptavidin–FITC. The surface biotinylation is shown in Figure 3b. The biotinylation process does not affect the viability of T cells, as detected by trypan blue dye exclusion test.

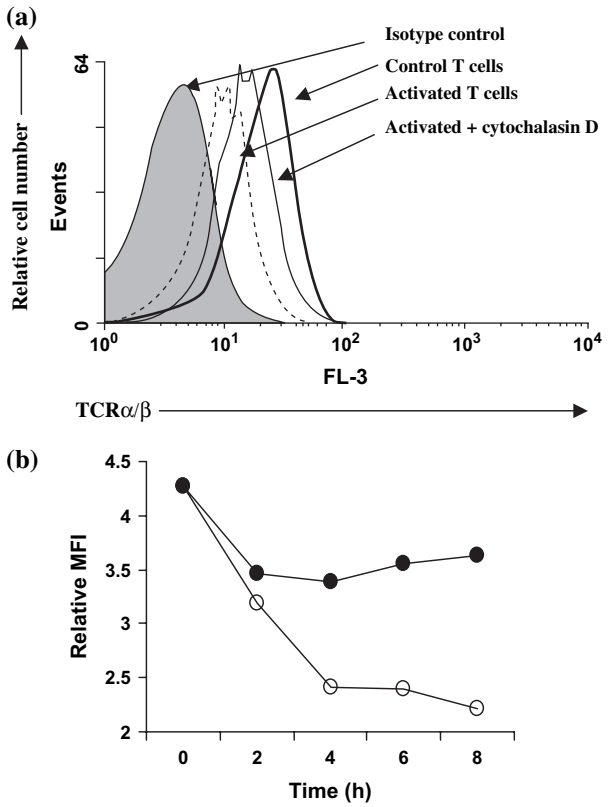

Figure 1 Downregulation of TCR by activated T cells. (a) Downregulation of surface TCR. Naive purified T cells were *in vitro* activated with PMA/ionomycin for 2 h in the presence or absence of cytochalasin D (20 μmol/L). Cells were stained with anti-TCRβ–PECy5 antibody and isotype control antibody. Data shown are representative of three independent experiments. (b) Kinetics of TCR downregulation. T cells were *in vitro* activated with PMA/ionomycin for the indicated time points and stained with anti-TCRα/β-FITC and analysed by FACS, and mean fluorescence intensity (MFI) were calculated. Relative MFI were calculated by dividing experimental sample MFI with isotype control MFI. Data shown are representatives of two independent experiments. ●, control T cells; ○, activated T cells.

After activation, cells were lysed, and TCR molecules were immunoprecipitated using antimouse TCRβ antibody and protein G-sepharose. The immunoprecipitates were resolved by SDS–PAGE and transferred to nitrocellulose membrane and probed with streptavidin–HRP. The result shows that degradation of internalized TCR increases with time (Fig. 3c). This degradation is due to lysosomal enzymes because the immunoprecipitatable amount of the biotinylated TCR was greater in chloroquine-treated cells (Fig. 3c).

Interaction of idiotypic and anti-idiotypic T cells

Activated T cells express MHC class II[14,16,17,29] and costimulatory molecules,[30,31] and present antigen to cognate T cells.[16,17,29,32] We hypothesize that degraded TCR peptide can be processed and presented by MHC class II molecules to cognate CD4$^+$ T cells. To test this hypothesis, we generated Rinderpest virus nucleocapsid protein (N protein)-specific T cells (idiotypic T cells) by immunization of naive BALB/c mice. Anti-idiotypic T cells (reactive to TCR of idiotypic T cells) were generated by immunization of syngenic mice with apoptotic idiotypic T cells. This protocol generates anti-idiotypic T cells efficiently.[21] APC and cognate T cells come in close proximity, and the duration of interaction depends on the specificity and affinity of TCR and peptide-loaded MHC that is further enhanced by costimulatory molecules.[33]

To show that activated T cells process and present their TCR and interact with cognate T cells, cell adhesion assay of idiotypic and anti-idiotypic T cells was carried out. The schematic representation of this experiment is shown in Figure 4a. In this experiment, anti-idiotypic CD4$^+$ T cells were labelled with CFSE and mixed with unlabelled activated idiotypic T cells. The adhesion of these populations was monitored by fluorescence microscopy. The adhesion of idiotypic and anti-idiotypic cells is shown in Figure 4b. The total number of clumps of cells (with more than three cells per clump) present in a microscopic field and also the number of cell clumps with anti-idiotypic CD4$^+$ T cells, that is, with CFSE-labelled cells, were counted. The results show that activated idiotypic T cells show higher adhesion efficiency than non-specific T cells or activated anti-idiotypic T cells treated with chloroquine (Table 1). This adhesion is specific to TCR and MHC II as the adhesion of idiotypic and anti-idiotypic T cells was abolished after treatment of idiotypic T cells with anti-MHC class II antibody. The specificity of adhesion was tested using irrelevant idiotypic T cells specific for P protein in conjunction with N protein-specific anti-idiotypic T cells, which did not show any specific cell adhesion (Table 1).

Presentation of TCR peptide to cognate T cells

To show that downregulated TCR are processed by lysosomal enzyme and presented to other T cells, purified anti-idiotypic CD4$^+$ T cells were cultured with fixed idiotypic T cells. The result given in Figure 5a shows that activated idiotypic T cells trigger anti-idiotypic CD4$^+$ T cells. This trigger is idiopeptide specific as proliferation was not seen when cultured with activated control T cells or idiotypic T cells treated with chloroquine. Chloroquine inhibits processing and presentation of antigen in the lysosome by increasing the pH inside the lysosomal vehicle.[34] We have further reconfirmed this result by measuring IL-2 secretion by anti-idiotypic CD4$^+$ T cells after *in vitro* stimulation with activated idiotypic T cells (Fig. 5b). The presentation of idiotypic TCR is through MHC class II molecules, because IL-2 secretion was abolished in cells treated with anti-MHC class II antibody (Fig. 5a,b). These results show that activated idiotypic CD4$^+$ T cells process and present TCR through MHC class II molecules to cognate anti-idiotypic CD4$^+$ T cells.

Discussion

Anti-idiotypic T cells are implicated to be involved in the control of autoimmune diseases.[35,36] Immunization with idiotypic T cells has been shown to generate anti-idiotypic T-cell responses.[37,38] The idiotypic network was postulated to be operating in the control of autoimmune disease in animal

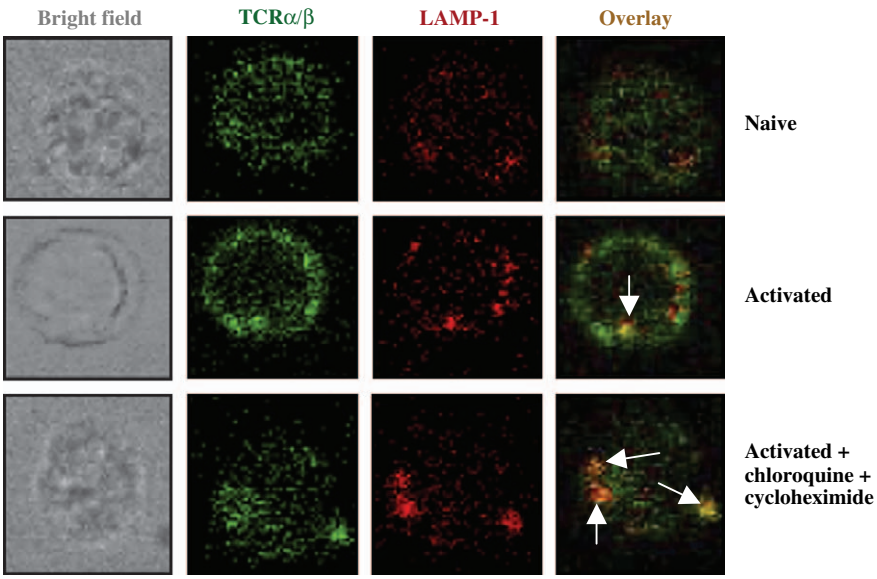

Figure 2 Colocalization of surface internalized TCR molecules with lysosomal-associated membrane protein-1 (LAMP-1). Purified naive T cells were *in vitro* activated with PMA/ionomycin for 3 h in the presence or absence of chloroquine (40 mmol/L) and cycloheximide (40 μmol/L). Cells were washed and incubated with a saturating concentration of anti-TCRα/β antibody (H57.597) on ice. Cells were washed, and intracellular staining was carried out using purified rat antimouse LAMP-1/antirat-tetramethylrhodamine B isothiocyanate antibody and anti-TCRα/β-FITC antibody. Cells were seen by confocal microscopy (×100 objective). The white arrow shows the colocalization of TCR and LAMP-1 molecules.

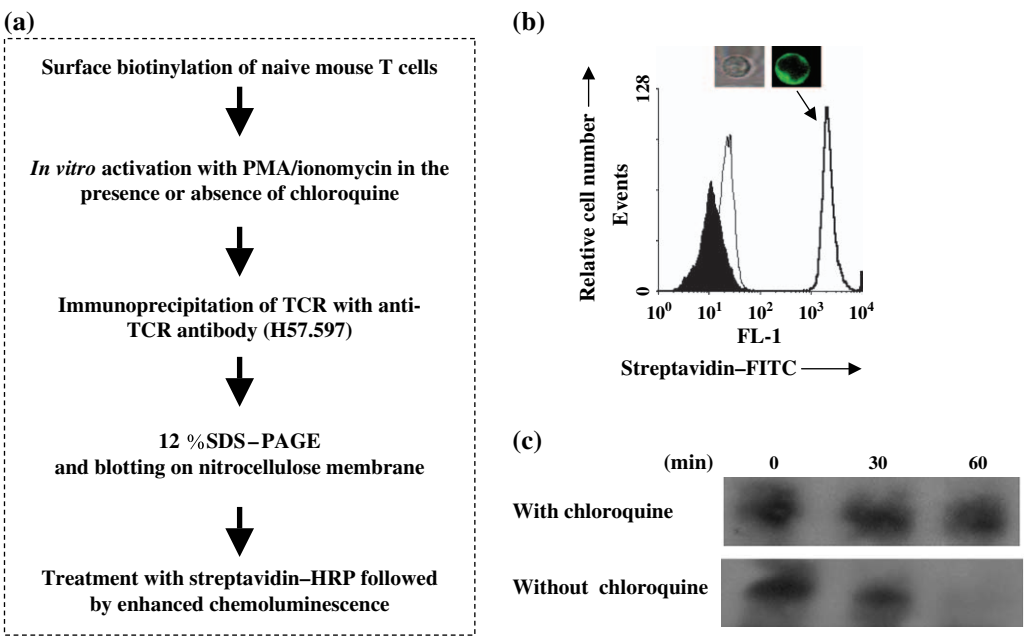

Figure 3 Degradation of surface TCR molecules. (a) Schematic representation of detection of degradation of surface TCR. (b) Surface biotinylation of T cells. Purified naive T cells were surface biotinylated with *N*-hydroxysuccinimido biotin. Biotinylation was detected after staining with streptavidin–FITC by FACS. Filled histogram represents unstained cells, thin line histogram represents the staining of nonbiotinylated cells with streptavidin–FITC, and thick line histogram represents biotinylated stained cells with streptavidin–FITC. (c) Degradation of TCR. Purified naive T cells were surface biotinylated and activated with PMA/ionomycin for indicated time points in the presence or absence of chloroquine (40 mmol/L). Cells were washed and lysed. TCR were immunoprecipitated using anti-TCRβ antibody and protein G-sepharose. The immunoprecipitate was analysed on 12% SDS–PAGE gels and probed with streptavidin–horseradish peroxidase (HRP), and the blot was developed using an enhanced chemiluminescence (ECL) kit.

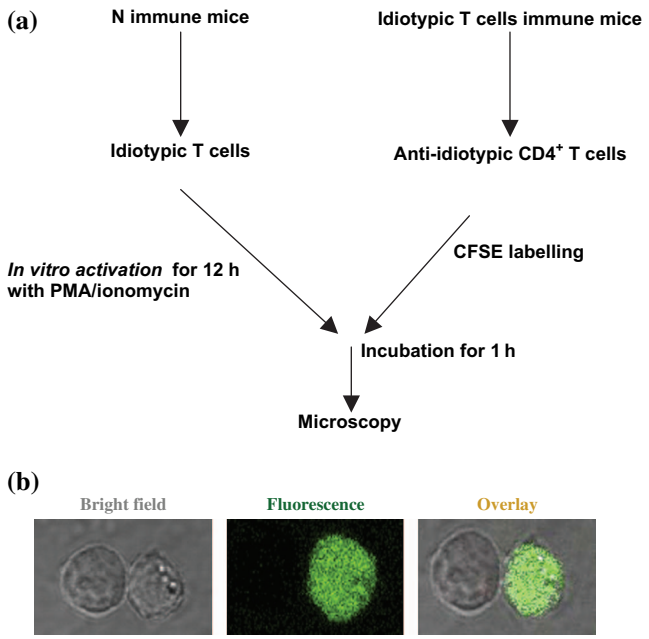

Figure 4 Interaction of idiotypic and anti-idiotypic T cells. (a) Schematic presentation of cell adhesion assay. (b) Purified activated anti-idiotypic T cells were mixed with carboxyfluorescein diacetate succinimidyl ester (CFSE)-labelled idiotypic T cells in a 1:4 ratio in complete RPMI medium. Cells were incubated for 30 min at 37°C with intermittent tapping. Cells were placed on glass slide, and cell adhesion was observed under fluorescence microscope.

models and in patients.[19,36,38,39] However, the exact mechanism of generation of anti-idiotypic T cells, which requires presentation of TCR peptides by MHC, was not defined. For generation of anti-idiotypic T cells, we postulate two possible mutually nonexclusive pathways. First, it is known that approximately 90% of antigen-specific T cells (idiotypic T cells) die at the contraction phase of the immune response.[40,41] These dead cells are removed by phagocytic cells (e.g. macrophages and dendritic cells). These phagocytic cells can process TCR and present the idiopeptide (peptide derived from the variable region of TCR) through MHC class II or by cross-presentation by the MHC class I pathway. Presentation of idiopeptide can trigger anti-idiotypic T cells. It is now clear that presentation of antigen from apoptotic cells leads to an immune response rather than to immunosuppression.[42–45] Another possible way for generation of anti-idiotypic T cells is through T cells functioning as APC. Activated T cells are known to present antigen to other T cells.[16,27] It is reported that approximately 30% of newly synthesized proteins are formed as defective ribosomal products (DRiP) during the normal protein synthesis process.[46,47] Thus, it is possible that TCR may also be synthesized as DRiP in T cells, which could be processed and presented by MHC class I molecules to provide a trigger for anti-idiotypic $CD8^+$ T cells.[48] However, for the $CD4^+$ T-cell response, these peptides must be presented through MHC class II molecules by T cells. For this presentation, the T cells must express MHC class II molecules and self-TCR should be processed and presented. Activated T cells have been shown

Table 1 Cell adhesion of anti-idiotypic $CD4^+$ cells with idiotypic T cells

Idiotypic-α-idiotypic T-cell interaction	Adhesion efficiency (%)
With N-specific α-idiotypic $CD4^+$ T cells	
Control T cells	10.3 ± 3.8
In vitro activated control T cells	9.3 ± 2.5
Idiotypic T cells	8.1 ± 1.8
In vitro activated idiotypic T cells	31.6 ± 1.9
In vitro activated idiotypic T cells + chloroquine	8.0 ± 1.2
In vitro activated idiotypic T cells + α-MHC class II antibody	7.1 ± 2.4
With P-specific α-idiotypic $CD4^+$ T cells	
Idiotypic T cells	8.8 ± 3.2
In vitro activated idiotypic T cells	6.8 ± 0.8

The adhering cell clumps were counted and adhesion efficiency was calculated as given in the Materials and methods. Data are representative of two independent experiments.

to express MHC class II molecules in humans,[17,27,32] in rats[14] and in mice by acquisition and endogenous synthesis.[15,16] T cells have also been shown to express costimulatory molecules.[31,37] Processing and presentation of antibody molecules by B cells have been shown earlier[49,50] and have been proposed to generate anti-idiotypic T-cell responses.[20,51] Recently, we have shown that booster immunization of antigen-specific anti-idiotypic T cells leads to the generation of antigen-specific memory response in antigen-primed animals.[21] Furthermore, we have shown that phagocytic cells process and present idiopeptides from idiotypic to anti-idiotypic T cells. In the present work, we have examined whether the surface TCR molecules of T cells are internalized, processed in the endosomal/lysosomal compartment and presented to $CD4^+$ T cells in context with MHC class II molecules. We have tested downregulation of TCR in naive T cells after activation with PMA/ionomycin. The combination of PMA/ionomycin treatment has been shown to positively select $CD4^+$ T cells in thymocyte cultures.[52] Using a PMA/ionomycin activation system, we show that activated T cells downregulate their surface TCR, which is similar to the downregulation observed with cytokine-activated or other ligand-activated mouse and human T cells.[7,9,53–55] These surface TCR molecules in activated T cells enter lysosomal compartments. Peptides are generated in the highly acidic environment of lysosomes by proteolytic cleavage of proteins.[55] We show that PMA/ionomycin-activated T cells internalize their TCR, as evidenced by colocalization of surfaced-labelled TCR and LAMP-1 and degrade them in the lysosomal compartment; the process is chloroquine sensitive.

Interaction of T cells through their TCR with cognate peptide presenting MHC class II molecules on the antigen presenting T cells (T-APC) leads to the formation of multicellular complexes, which can be measured by cell adhesion assay. Using cell adhesion assay, we showed that activated idiotypic T cells efficiently adhere to specific anti-idiotypic $CD4^+$ T cells (31% cell adhesion efficiency as compared to 10% for non-specific adhesion). Inhibition of antigen processing by chloroquine or treatment of T-APC (activated idiotypic T cells) with anti-MHC class II antibody decreases cell adhesion. The above finding indicates that antigen processing

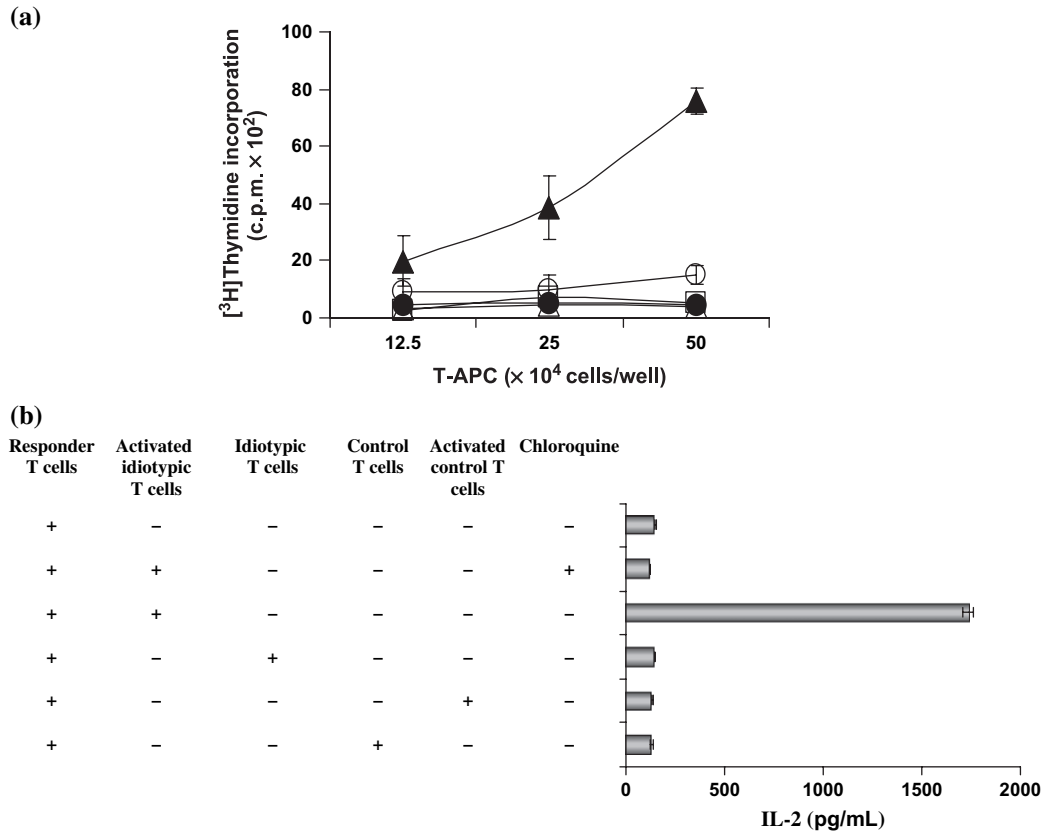

Figure 5 Presentation of idiopeptide by T cells. (a) Purified idiotypic T cells were activated with PMA/ionomycin for 12 h. Cells were fixed with 0.2% paraformaldehyde and used as Antigen presenting cells (APC). Anti-idiotypic $CD4^+$ T cells were cocultured in the presence of a fixed number of idiotypic cells (4×10^5 cells/well) in 200 μL of complete medium and incubated for 72 h at 37°C. [^{3}H]thymidine (0.037 MBq/well) was added in the last 16 h of incubation. ●, Activated control T cells; ▲, activated idiotypic T cells; △, activated idiotypic T cells in presence of chloroquine; ○, non-activated idiotypic T cells; and □, activated idiotypic T cells with anti-MHC class II antibody (10 μg/mL). Data shown are ±SEM of triplicates. (b) Presentation of idiopeptide by idiotypic T cells to the anti-idiotypic $CD4^+$ T cells. *In vivo* generated purified idiotypic T cells were activated with PMA/ionomycin and used as APC after fixing. Fixed normal T cells or idiotypic T cells activated in presence of chloroquine (40 mmol/L) were used as control APC. Purified anti-idiotypic $CD4^+$ T cells (responder T cells) 1×10^5 cells/well were cocultured with fixed APC (4×10^5 cells/well) for 48 h in 200 μL of complete medium. Supernatants were collected, and IL-2 secretion was measured by ELISA. Data shown are the mean ± SEM of triplicates. c.p.m., counts per minute.

is involved and antigen presentation is through MHC class II molecules. Results of IL-2 secretion (Fig. 5b) show that only activated idiotypic T cells are able to present TCR peptide to cognate anti-idiotypic $CD4^+$ T cells, which are MHC class II dependent and chloroquine sensitive. The IL-2 secretion and proliferation results show that nonactivated anti-idiotypic T cells do not secrete IL-2. It is known that naive T cells lack costimulatory molecules,[30] and presentation of antigen in the absence of costimulatory molecules leads to the generation of tolerance and anergy in T cells.[56] Further experiments are necessary to explain the presence of TCR variable region peptide on the MHC class II molecule on the surface of T cells. This would help in understanding the mechanism underlying T-cell vaccination or treatment of T-cell lymphoma with TCR polypeptide.[19] Interaction of idiotypic and anti-idiotypic T cells also provide a mechanism for perpetuation of immunological memory as proposed earlier.[20] The presentation of antigen by MHC class II molecules by T cells may have very important consequence in control of lymphotropic viral infection. We have shown that activated mouse T cells express MHC class II molecules, and that they process and present antigen to cognate T cells.[16] Other researchers have reported similar results for human and rat T cells.[14,30] It appears that activated T cells form a diverse species, which have the ability to present antigen through class II MHC. T-cell activation in the living system is a continuous process due to both endogenous and exogenous antigen challenge. Therefore, generation of anti-idiotypic T cells directed against specific TCR idiopeptides would be an ongoing process. This phenomenon is important for maintenance of homeostasis of the immune system. It is tempting to speculate that anti-idiotypic T cells would also present their TCR to cognate T cells. If the idiopeptides of anti-idiotypic TCR generate peptidomimic of antigenic peptide, a self-sustaining cascade of idiotypic and anti-idiotypic T-cell responses are likely to be generated, which can carry forward immunological memory as proposed for B-cell memory.[20,51]

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