The functional role of class II-associated invariant chain peptide (CLIP) in its ability to variably modulate immune responses

Pratibha Chaturvedi, Ryan Hengeveld, Marc A. Zechel, Edwin Lee-Chan and Bhagirath Singh

Department of Microbiology and Immunology, and John P. Robarts Research Institute, University of Western Ontario, London, Ontario N6A 5C1, Canada

Keywords: antigen presentation, Ii chain peptides, MHC, T h cells

Abstract

During the process of class II MHC assembly and cell surface expression, the class II-associated invariant chain peptide (CLIP) is removed from the peptide-binding groove of MHC, a task mediated by H-2M. This allows binding and presentation of peptide epitopes. We have previously shown that exogenously added CLIP interferes with this process and down-regulates the cell surface expression of class II molecules. In this study, we explored the effect of exogenously added CLIP on antigen-specific immune responses. In vivo studies with CLIP and various peptide and protein antigens with different affinities for I-A\(^d\) molecules demonstrated that CLIP variably affects the T cell-mediated immune responses. Immunization with CLIP along with the antigen induced a shift from a T\(_{h1}\)- to T\(_{h2}\)-like response as determined by the cytokine profile and antibody isotype. These results suggest that the presence of exogenous CLIP can significantly influence the presentation of antigen by class II MHC molecules to CD4 T cells and thereby modulate immune responses. Exogenously added CLIP rapidly localized into the subcellular compartment of antigen-presenting cells where MHC class II molecules are present. We suggest that exogenous CLIP reduces the loading of peptides on the class II molecules, thus down-regulating MHC–peptide complexes on the cell surface. Alternatively, CLIP may bind to cell surface class II molecules and this complex is rapidly internalized resulting in reduced cell surface MHC class II expression. The reduced level of MHC–peptide complexes favors the activation of T\(_{h2}\) cells over T\(_{h1}\) cells. These results have implications in the regulation of immune responses, particularly the prevention of certain autoimmune diseases where T\(_{h1}\)-type responses are pathogenic and T\(_{h2}\)-type responses are protective.

Introduction

The class II MHC expressed by B cells, macrophages, dendritic cells and thymic epithelial cells is a heterodimeric molecule consisting of \(\alpha\) and \(\beta\) chains. These two chains, the \(\alpha\) and \(\beta\), are involved in binding peptides by virtue of highly polymorphic residues found at the N-terminal domains which line the peptide-binding groove of the MHC molecules (1). The class II MHC functions by binding self-peptides in the peptide-binding groove and by guiding the selection of CD4\(^+\) lymphocytes in the thymus. It is also involved in presenting peptide antigens to CD4\(^+\) cells in order to initiate immune responses.

After synthesis, the class II MHC molecule associates with the invariant chain (Ii) that serves to prevent premature binding of antigen in the peptide-binding groove in the endoplasmic reticulum (2). In the endosomes the Ii undergoes degradation and a peptide fragment (85–101) called class II-associated invariant chain peptide (CLIP) remains in the peptide-binding groove (3,4). CLIP has also been shown to prevent peptide binding (5,6) and is, therefore, assumed to be tightly bound to the binding groove of the MHC. For the peptide antigens to bind to the class II MHC it is necessary to remove CLIP from the peptide-binding groove. The removal of CLIP from the peptide-binding groove is a task facilitated by H-2M.

H-2M plays a vital role in the removal of CLIP and for allowing peptide epitopes to bind to the class II MHC molecules. In
the absence of H-2M, CLIP would prevent the binding of antigens in the binding groove and their subsequent presentation on the cell surface (7). We have previously shown that saturating the endosomal compartment with exogenous CLIP down-regulates the surface MHC class II expression and inhibits the ability of antigenic epitopes to bind to class II MHC (15).

The present study was undertaken to analyze the effect of exogenously added CLIP on the activation of T cells and on the Th1 cell subsets generated. Using confocal microscopy we show that CLIP is rapidly internalized by the antigen-presenting cells (APC) and it localizes into the subcellular compartment where class II MHC molecules are present. Exogenous CLIP may have a significant effect on the density of peptides being presented at the surface of the APC leading to modulation of T cell response. We hypothesized that since CLIP down-regulates MHC expression (15), the density of peptide–MHC complexes at the surface may be altered such that a Th2 rather than a Th1 response may be induced to the peptide–MHC complexes on the cell surface (7). We have previously shown that saturating the endosomal compartment with exogenous CLIP down-regulates the surface MHC class II expression and inhibits the ability of antigenic epitopes to bind to class II MHC (15).

Antigen

CLIP(85–101), FITC-CLIP(85–101), OVA(323–339), K1A2, K3 and K4 peptides were prepared by the Merrifield solid-phase technique on a ABI 431A Peptide Synthesizer (Applied Biosystems, Mississauga, Ontario, Canada) as described earlier (14,15). The peptides were purified by HPLC on a C18 reverse-phase semi-preparative SynChropak RP-P column (SynchroM, Linden, IN), using a linear gradient from water to acetonitrile (1.37% acetonitrile/min). For functional assays, peptides were dissolved in saline by adjusting pH to 7.2 with 0.1 N NaOH and were sterilized by filtration through a 0.22 μm filter. Abbreviations used for amino acids: K, lysine; E, glutamic acid; Y, tyrosine; A, alanine. Purified protein derivative (PPD; Statens Serum Institut Tuberculin Department, Copenhagen, Denmark) was used at a concentration of 40 μg/ml as a positive control in adjuvant [complete Freund’s adjuvant (CFA)]-primed mice.

Methods

Mice

BALB/c female mice (8–10 weeks old) used in this study were purchased from Jackson (Bar Harbor, ME).

Antigen

CLIP(85–101), FITC-CLIP(85–101), OVA(323–339), K1A2, K3 and K4 peptides were prepared by the Merrifield solid-phase technique on a ABI 431A Peptide Synthesizer (Applied Biosystems, Mississauga, Ontario, Canada) as described earlier (14,15). The peptides were purified by HPLC on a C18 reverse-phase semi-preparative SynChropak RP-P column (SynchroM, Linden, IN), using a linear gradient from water to acetonitrile (1.37% acetonitrile/min). For functional assays, peptides were dissolved in saline by adjusting pH to 7.2 with 0.1 N NaOH and were sterilized by filtration through a 0.22 μm filter. Abbreviations used for amino acids: K, lysine; E, glutamic acid; Y, tyrosine; A, alanine. Purified protein derivative (PPD; Statens Serum Institut Tuberculin Department, Copenhagen, Denmark) was used at a concentration of 40 μg/ml as a positive control in adjuvant [complete Freund’s adjuvant (CFA)]-primed mice.

Antibody

Anti-I-A6 mAb MKD.6 (15) and TRITC–goat anti-mouse-FC (Sigma, St Louis, MO) were used in confocal microscopy.

Immunization and T cell proliferation assay

BALB/c mice were immunized with 50 μg of K3, K4 or K1A2 emulsified in CFA (Sigma) in both hind footpads. One group of mice was immunized with either of the peptides in the presence or absence of 50 μg CLIP or OVA(323–339) peptide. One group of mice was immunized with 50 μg of OVA protein (Sigma) along with 50 μg CLIP, or a control peptide K1A2. After 10 days, popliteal lymph nodes were removed and a single-cell suspension was prepared. The cells were then cultured in 96-well flat-bottom plates (Becton Dickinson, Mississauga, ON) at 2 × 10^5 cells/well in the presence or absence of the peptide (50 μg/ml) in 200 μl of culture medium (RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% FCS (Bockneck, Rexdale, Ontario, Canada), 10 μM HEPES, 2 mM L-glutamine, 5 × 10^-5 M 2-mercaptoethanol and 1 U/ml penicillin/streptomycin). After 3 days, cultures were pulsed with 1 μCi/well of [3H]thymidine (NEN Du Pont, Boston, MA) for 16–20 h. Incorporation of [3H]thymidine was measured using a liquid scintillation counter (LKB, Gaithersburg, MD). Data is presented for one antigen concentration; however, results were obtained with various concentrations of the peptides or CLIP as we previously reported (8,15).

Cytokine assays

Cells (2 × 10^6) from immunized mice were cultured in 24-well flat-bottom plates (Corning Glass Works, Corning, NY) in 2 ml medium in the presence or absence of the peptides K3, K4, K1A2 or OVA (50 μg/ml). Supernatants were collected at different time periods and assayed for different cytokine contents. IL-4 was assayed using IL-4-dependent CT4.S cells (kindly supplied by Dr B. Chan, Robarts Research Institute, London, Ontario, Canada). CT4.S cells were cultured with supernatant for 30 h. [3H]Thymidine (1 μCi/well) was added and cultured for an additional 18 h. Incorporation of [3H]thymidine was assayed as described above. A standard curve was obtained using IL-4-containing supernatant (starting at the highest concentration of 100 U/ml) from murine rIL-4 cDNA-transfected X63Ag8-653 myeloma cells (8). Supernatants were also assayed for IFN-γ using cytokine-specific ELISA (PharMingen, Mississauga, Ontario, Canada). A standard curve was obtained using recombinant IFN-γ (starting at highest concentration of 40 ng/ml).

Antibody response

Mice were immunized with 50 μg K3, K4 or K1A2 in 50 μl saline emulsified in 50 μl of CFA into one hind footpad. One
group of mice was injected with peptide plus CLIP (50 µg each) emulsified in CFA. After 2 weeks, a second injection of the respective peptide (50 µg) or peptide plus CLIP (50 µg each) emulsified in incomplete Freund’s adjuvant (IFA) was given i.p. Blood was collected at different time intervals after the second injection and serum was separated. ELISA plates (Becton Dickinson) were coated with the peptide, and the isotype of antibodies generated against K3, K4 and K1A2 was detected by using goat anti-mouse IgG1 or IgG2a antibody (Caltag, San Francisco, CA).

Confocal microscopy
To determine uptake and localization of CLIP(85–101), a B cell hybridoma, TA3 cells, were incubated for various times with FITC–CLIP (100 µg/ml) at 37°C. Subsequently, cells were washed twice in PBS and incubated at room temperature on poly-L-lysine-coated glass coverslips (Sigma) for 15 min. Attached cells were fixed in 2% paraformaldehyde for 10 min at room temperature and washed in PBS. Cells were directly mounted on glass slides using Vectashield (Vector, Burlingame, CA) and visualized for fluorescence. For co-localization experiment, cells were attached to the glass coverslips and fixed in 2% paraformaldehyde. These cells were then either permeabilized by incubating in −20°C acetone for 2 min or left unpermeabilized. Cells were then incubated with primary mAb, MK-D6 (anti-I-A^d^, 1:250) or FITC–CLIP (100 µg/ml) for 35 min at room temperature and washed 4 times with PBS over 5 min. The cells incubated with anti-I-A^d^ antibody were incubated with secondary antibody, TRITC–goat anti-mouse-Fc (1:5000) for 30 min at room temperature and washed with three changes of PBS over 5 min. Stained cells were then mounted on glass slides as above and visualized for fluorescence on a Carl Zeiss confocal microscope.

Results

Exogenously added CLIP down-regulates the proliferative response to OVA protein
As protein antigens require processing before they are able to bind to MHC we examined if immunization with CLIP would modify the immune response to protein antigen in vivo. Mice were immunized with OVA protein (50 µg/hind footpad) with or without CLIP (50 µg/hind footpad) emulsified in CFA in both the hind footpads. After 10 days, lymph nodes were harvested and T cell proliferation was assayed. The results presented in Fig. 1 show that addition of CLIP during immunization down-regulated the proliferative response of mice to OVA protein. Possibly, exogenously added CLIP interferes with the binding of intracellularly processed peptide fragments of OVA to MHC molecules, decreasing the presentation to T cells, thus resulting in down-regulation of the proliferative response. Next we examined the effect of immunization with CLIP on the generation of T_{h1} and T_{h2} responses to OVA. The results presented in Fig. 2 show a higher level of IL-4 secretion upon immunization with OVA plus CLIP and a decreased level of IFN-γ suggesting a shift from a T_{h1}- to T_{h2}-type response. On the other hand, the control peptide K1A2 used in place of CLIP did not have any effect on the outcome of the immune response to OVA protein. These results suggest that CLIP can modulate the T cell response to protein antigens.

Exogenously added CLIP inhibits the in vivo response to peptides
We have previously reported that exogenous CLIP binds poorly to cell surface MHC class II molecules (15) but it down-regulates the surface expression of class II molecules. To determine the effect of CLIP on the presentation of peptide antigens by APC, BALB/c mice were immunized with CLIP or a control OVA(323–339) peptide (50 µg/footpad) along with three related homologous peptide antigens K3, K4 or K1A2 (50 µg/footpad). Peptide K3 has low affinity and K1A2 has high affinity, while K4 has an intermediate affinity for I-A^d^ molecules (6). Ten days later, lymph nodes were harvested and T cell proliferation was assayed. Immunization with the peptides in the presence of CLIP down-regulated the proliferative response compared to immunization with peptides alone (Fig. 3). However, the effect of CLIP was more pronounced when OVA protein was used. As expected, addition of the OVA(323–339) peptide that binds to I-A^d^ in place of CLIP also resulted in a reduction in the response. This suggests that both CLIP and OVA(323–339) peptide compete with K3, K4 or K1A2 peptides for binding to MHC, resulting in inhibition of the presentation of the antigens to T cells and subsequent down-regulation of the immune response.

Exogenously added CLIP shifts the immune response toward T_{h2}
We have reported earlier that high-affinity peptide K1A2 induces the generation of a T_{h1}-type response while low-affinity peptide K3 induces a T_{h2}-type response. The peptide
Modulation of T cell subset activation in vivo by CLIP

K4 with intermediate affinity induces both Th1 and Th2 responses (8). To analyze whether down-regulation of antigen presentation by CLIP alters the differentiation of Th1 and Th2 cells in response to peptide antigens, mice were immunized with peptides in the presence of CLIP or OVA peptide. After 10 days, draining lymph nodes were harvested and cells were cultured with peptide antigens. Supernatants were collected and assayed for the presence of IL-4 and IFN-γ as described in Methods. A standard curve was obtained using recombinant IFN-γ starting with a highest concentration of 40 ng/ml. A standard curve was obtained using IL-4 starting with a highest concentration of 100 U/ml. IFN-γ was assayed using cytokine-specific ELISA as described in Methods. A statistically significant difference was observed when *P < 0.02, **P < 0.01, and ***P < 0.001.

Exogenously added CLIP shifts the isotype of peptide-specific antibodies from IgG2a to IgG1

Mice were immunized with K3, K4 or K1A2 peptides (50 µg/footpad) with or without CLIP (50 µg/footpad) emulsified in CFA. Two weeks later mice were given a second injection of peptides (100 µg) emulsified in IFA with or without CLIP (100 µg). Mice were bled 2 weeks after the second injection, and the serum was then assayed for the presence of IgG1 and IgG2a antibodies. The peptide K3 induces very low levels of both IgG1 and IgG2a isotype antibodies. Addition of CLIP to the peptide increased the levels of IgG1 antibodies indicating an increase in the Th2 response. On the other hand, immunization with K4 and CLIP did not seem to have an effect on IgG1 response but down-regulated the IgG2a response, suggesting down-regulation of the Th1-type response while the Th2 response is maintained. The third peptide, K1A2, displayed the biggest shift in antibody response upon addition of CLIP. The IgG1 antibody response increased when CLIP was added, whereas the IgG2a response was significantly reduced, suggesting a shift from a strong Th1 to a Th2 response (Fig. 5). These results confirm the results obtained from cytokine studies.
Modulation of T cell subset activation in vivo by CLIP

Fig. 4. Effect of immunization with CLIP on the generation of T<sub>H1</sub>- and T<sub>H2</sub>-type cells. BALB/c mice were immunized with 50 µg/footpad of either of the peptides in the presence or absence of CLIP (50 µg/footpad) emulsified in CFA. For control, OVA(323–339) peptide was used in place of CLIP. After 10 days, draining lymph nodes were harvested and cells were cultured in the presence of the respective peptide (50 µg/ml). Culture supernatants were collected after 24 h, and tested for the presence of IL-4 and IFN-γ. For IL-4 assay, CT4.S cells were cultured with supernatant for 30 h. [3H]Thymidine (1 µCi/well) was added and cells were cultured for an additional 18 h. Incorporation of [3H]thymidine was measured as described in Methods. A standard curve was obtained using IL-4 starting at a highest concentration of 100 U/ml. IFN-γ was assayed using cytokine-specific ELISA as described in Methods. A standard curve was obtained using recombinant IFN-γ starting with a highest concentration of 40 ng/ml. For IL-4 assay, statistically significant differences where *P < 0.05, **P = 0.02 and ***P = 0.05; for IFN-γ assay *P < 0.02 and **P < 0.01.

CLIP co-localizes in an intracellular, MHC-containing endosomal compartment

In a previous study we have shown that CLIP down-regulates the cell surface expression of MHC class II molecules (15). To determine how CLIP down-regulates the MHC expression, uptake of CLIP by TA3 cells and its localization inside the cell was examined using confocal microscopy. We postulated that exogenous CLIP was localizing in the MHC-containing compartments where peptide-loading takes place. Based on this assumption we performed co-localization experiments using permeabilized or unpermeabilized TA3 cells. Cells were incubated with FITC-CLIP, anti-I-A<sup>d</sup> antibody or both and visualized under a confocal microscope. The images presented in Fig. 6 show that both CLIP and I-A<sup>d</sup> antibody bind to the cell surface of unpermeabilized cells. The binding of anti-I-A<sup>d</sup> antibody seems to be more intense compared to binding of CLIP that appeared rather diffused. Incubation of unpermeabilized TA3 cells with both FITC-CLIP and anti-I-A<sup>d</sup> did not show appreciable co-localization on the surface. However, when permeabilized cells were used, CLIP appears to localize in well-defined subcellular vesicular structures. The permeabilized cells stained for both I-A<sup>d</sup> and CLIP demonstrate that CLIP co-localized with I-A<sup>d</sup> (Fig. 6). This suggests that exogenously added CLIP is internalized and is localized in the MHC-containing subcellular vesicles. To further confirm that exogenous CLIP is internalized, TA3 cells were incubated with FITC-labeled CLIP for various times and visualized directly by confocal microscope (Fig. 7). It was observed that CLIP binds to the surface initially and is internalized in a time-dependent fashion localizing in well-defined subcellular vesicles as observed in the co-localization experiment (Fig. 6). Over a 2 h time course exogenous CLIP is internalized through cell surface-associated subcellular vesicles and localizes with class II MHC as observed in Fig. 6.
Fig. 6. Co-localization of MHC class II molecules and exogenously added CLIP in the cell. TA3 cells (B cell hybridoma) were incubated with FITC-labeled CLIP (100 µg/ml) at 37°C for 30 min. Subsequently, cells were adhered to glass coverslips and fixed as described in Methods. Cells were washed, permeabilized by incubating in −20°C acetone for 2 min or left unpermeabilized. Cells were then incubated with monoclonal anti-I-A<sup>d</sup> antibody (MKD.6; 1:250) for 35 min at room temperature. Cells were washed and incubated with secondary antibody, TRITC–goat anti-mouse-Fc (1:5000), for 30 min at room temperature. Cells were washed, mounted on glass slides and visualized for fluorescence using a Carl Zeiss confocal microscope.

Discussion

MHC molecules need a transport-competent conformation for effective post-endoplasmic reticulum transport. Class II MHC molecules assemble as a stoichiometric complex with trimers of the type II integral membrane glycoprotein Ii (16). The interaction with the Ii chain: (i) contributes to efficient stable association of the class II α and β subunits in the endoplasmic reticulum (17,18), (ii) promotes the transport of class II α/β heterodimers from the endoplasmic reticulum through the Golgi complex (17,19–22), and (iii) inhibits the binding of other ligands (peptides) to the class II molecules (6,23,24). The α/β–Ii complex undergoes extensive modification during its transport through the Golgi to endosomes where the tail portion of the Ii is degraded.

This leaves CLIP(85–101) in contact with the α/β heterodimer (3,4). CLIP has been shown to prevent peptide binding to the major groove of the MHC class II molecule (5,6). Therefore, for efficient peptide binding to MHC and subsequent presentation to T cells, CLIP needs to be removed from the peptide-binding groove by H-2M molecules.

We investigated the functional role of exogenously added CLIP in the modulation of the immune response and its localization in the cell. We have reported earlier that saturation of the endosomal compartment with exogenous CLIP results in reduced class II MHC expression and down-regulation of T cell responses (15). It has been reported by several workers during the past few years that CLIP binds to class II MHC within the antigen-binding groove (25–28). However, there are no reports concerning the functional role of exogenous CLIP in the modulation of the immune response.

Experiments were done to determine if exogenously added CLIP is internalized to affect the immune responses. Our data shows for the first time that exogenously added CLIP is internalized and it co-localizes in MHC class II-containing compartments. It is not clear from our studies where in the endocytic pathway FITC–CLIP is localized, but our data show that it is internalized in a time-dependent fashion and is localized in well-defined subcellular vesicles as opposed to diffuse patches on the cell surface. It has been reported recently that endogenous CLIP localizes with HLA-DR in multimembranous compartments that are positioned late in the endocytic pathway and are the most likely site where CLIP is exchanged for peptide (29). It is possible that
exogenous CLIP also localizes in compartments late in the endocytic pathway but further experiments are needed to confirm this. Although our confocal data showed that FITC–CLIP binds to the cell surface, there was no appreciable co-localization of CLIP with MHC class II on the cell surface. Using a human B cell line, it has been shown that a large population of MHC class II–II complexes reaches endosomes by rapid internalization from the cell surface (30). It is possible that the lack of co-localization on the cell surface may be due to the internalization of the MHC class II–CLIP complexes. The down-regulation of class II MHC expression observed by addition of exogenous CLIP may also be due to the rapid recycling of the MHC–CLIP complexes from the cell surfaces by internalization into endosomes.

We further investigated the effect of CLIP on the immune response induced by various peptide antigens. Three peptides with different affinities for class II MHC were used. We have reported earlier that the peptide K3 with low affinity for class II MHC induces a Th2-type response while K1A2 with high affinity for class II MHC induces a Th1-type response. The peptide K4 with intermediate affinity induces both a Th1- and Th2-type response (8). Immunization of mice with K3, K4 and K1A2 along with CLIP significantly down-regulated the proliferative response towards each of the peptides. Immunization with a control peptide OVA(323–339) in place of CLIP also inhibited the response but the inhibitory effect of CLIP was more pronounced. These results suggest that both CLIP and OVA peptides compete with the antigens for binding to class II MHC molecules. This was confirmed by the fact that T cells from OVA plus K3-, K4- or K1A2-immunized mice proliferated in response to OVA peptide also (data not shown).

Immunization of mice with peptides along with CLIP altered the cytokine profile induced by these peptides. An increase in IL-4 secretion was detected in response to all three peptides while IFN-γ production was down-regulated, suggesting a shift towards a Th2-type response. This was confirmed by the antibody response to these peptides, IgG1 corresponds to Th2 and IgG2a corresponds to a Th1-type response (13). Immunization with K3 plus CLIP up-regulated the IgG1 isotype of antibodies suggesting a stronger Th1 response. Immunization with K4 plus CLIP down-regulated the IgG2a response while maintaining the IgG1 response suggesting down-regulation of Th1 responses. On the other hand, immunization with K1A2 plus CLIP resulted in a significant increase in IgG1 response and down-regulation of IgG2a, again suggesting a shift from a Th1- to Th2-type response. Immunization with peptides plus OVA(323–339) resulted in up-regulation of IFN-γ secretion by K4- and K1A2-primed cells. There was a decrease in IL-4 secretion by K3-primed cells when mice were immunized with K3 plus OVA(323–339) but increase in IFN-γ secretion was not significant. Although both the peptides seem to down-regulate the immune responses, the mechanism of their action could be different. As discussed earlier, exogenously added CLIP down-regulates the expression of class II MHC on the cell surface by rapid internalization of MHC–CLIP complexes (30). It is possible that after internalization into endosomal compartments CLIP dissociates from the MHC for loading of the peptides. However, saturation of the peptide-loading compartment with exogenous CLIP may inhibit the binding of peptides by competing with the antigen for binding to class II MHC thus resulting in the decreased antigen presentation. The low ligand density on the surface of APC favors the generation of Th1, Th2-type cells. It has been previously demonstrated that ligand density, along with the type of APC and particular epitope presented, is an important factor in determining whether a Th1- or Th2-like response is generated (8,9). Being an antigenic peptide, OVA(323–339) up-regulates the expression of MHC on the cell surface. Therefore, the down-regulation in proliferative response observed after immunization with K3, K4 or K1A2 peptide in the presence of OVA(323–339) could be due to the competition between these peptides for binding to MHC and subsequent presentation to T cells. The high ligand density on the surface of APC results in the generation of a Th1-type response (8,9). Indeed, OVA(323–339) peptide induces a Th1-type response when mice are immunized with this peptide alone (data not shown). Generation of Th1 response by OVA(323–339) peptide may explain the up-regulation of IFN-γ secretion after immunization with peptides in the presence of OVA(323–339) peptide. There was a decrease in IL-4 secretion by K3-primed cells after immunization with K3 plus OVA(323–339) peptide but there was no effect on IL-4 secretion by K4-primed cells. One possible explanation for this could be that immunization with OVA(323–339) up-regulates the class II MHC expression leading to increased presentation of K3 resulting in a shift toward a Th1-type response. We have shown earlier that increasing the immunization dose of K3 compensates for its low affinity and shifts the response towards Th1 (8). Another possibility is that due to the lower affinity of K3 it does not compete with OVA(323–339) peptide for binding to MHC as efficiently as K4, resulting in a lower K3-specific response leading to decreased IL-4 secretion. Although there is a down-regulation of IL-4 secretion by K3-primed cells, a very small amount of IFN-γ was detected in these cell cultures suggesting no significant shift in the response towards Th1. Thus, CLIP down-regulates the immune response and shifts the response toward Th2 type but OVA(323–339) peptide down-regulates the immune response without altering the Th1/Th2 balance.

Protein antigens require processing before presentation to T cells. We investigated the effect of CLIP on the presentation of OVA protein. It was observed that CLIP down-regulated the proliferative response of T cells to OVA. A change in the cytokine profile was also observed. There was an increase in the levels of IL-4 secretion and decrease in IFN-γ production. However, the control immunogenic peptide K1A2 did not have any effect on the response to OVA protein. There are a few possible explanations for the effect of CLIP on immune responses to protein and peptide antigens. First, exogenously added CLIP is internalized and competes with peptide antigens or peptide fragments generated after the processing of proteins for binding to MHC in the peptide-loading compartment. Second, the CLIP–MHC complexes on the cell surface may be structurally different from the MHC class II molecules occupied by other peptides and, therefore, may not present antigen efficiently resulting in reduced response. It has been reported recently that H-2M mutant mice express normal levels of MHC class II but these are structurally distinct from the compact SDS-resistant complexes expressed by wild-
type cells and are mostly bound by CLIP (31,32). The APC from the H-2M mutant mice are not efficient in presenting protein or peptide antigens (33). This may be the case when the peptide-loading compartment is saturated with exogenously added CLIP in our studies resulting in the down-regulation of the immune response.

The precise mechanism of exogenous CLIP-mediated down-regulation of MHC expression and subsequent modulation of T cell responses needs to be further elucidated. We postulate that this could involve classical and novel endocytic compartments in the APC. In mouse cells class II vesicles (CIIV) represent a novel endocytic compartment involved in antigen processing and class II peptide loading. In human cells, the MHC class II-enriched compartment (MIIC) represents a lysosome-like endocytic compartment that appears to be involved in this process. Recently, a subcellular compartment morphologically and immunologically similar to human MIIC has been identified in murine B cells (34). These MIIC-like vesicles represent a population of class II^+ late endosomes, are distinct from CIIV and appear to be the major repository of DM molecules in these cells. The relationship between CIIV, MIIC and classical endosomes and lysosomes remains to be determined. We postulate that one or more of these compartments are involved in internalization of CLIP and loading of the peptides.

CLIP is able to down-regulate the proliferative response to various antigens without eliminating it. CLIP is also able to alter the nature of the T_h1 subset populations and as a result change the way in which the immune system responds to antigens. The T_h2 subset is important for the generation of humoral immune responses, whereas the T_h1 subset plays a critical role in generating delayed-type hypersensitivity immune responses (11). The T_h1 subsets are often associated with autoimmune conditions and play an important role in causing inflammatory disorders. Although immunogenic peptides can affect the response to an antigen by competing for presentation and mounting an immune response, CLIP being non-immunogenic can be used to modulate the response to any antigen without concern for an unnecessary response. Moreover, CLIP can modulate the response of protein antigens very effectively while immunogenic peptide used in our study did not have any effect. Therefore, CLIP may be an effective tool to modulate responses to both protein and peptide antigens, and shift them from a pathogenic disease causing a T_h1 immune response towards a T_h2 response that is often associated with protection in a number of autoimmune diseases involving T_h1 cells.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>antigen-presenting cell</td>
</tr>
<tr>
<td>CFA</td>
<td>complete Freund's adjuvant</td>
</tr>
<tr>
<td>CIIV</td>
<td>class II vesicles</td>
</tr>
<tr>
<td>CLIP</td>
<td>class II-associated invariant chain peptide</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>IFA</td>
<td>incomplete Freund's adjuvant</td>
</tr>
<tr>
<td>K</td>
<td>invariant chain</td>
</tr>
<tr>
<td>K1A2</td>
<td>EYKEYAYEAAYEA</td>
</tr>
<tr>
<td>K3</td>
<td>EYKEYAYAEYAYEA</td>
</tr>
<tr>
<td>K4</td>
<td>EYKEYAYAEYAYAE</td>
</tr>
<tr>
<td>MIIC</td>
<td>MHC class II-enriched compartment</td>
</tr>
<tr>
<td>PPD</td>
<td>purified protein derivative of Mycobacterium tuberculosis</td>
</tr>
<tr>
<td>OVA</td>
<td>ovalbumin</td>
</tr>
<tr>
<td>TRITC</td>
<td>tetramethylrhodamine B isothiocyanate</td>
</tr>
</tbody>
</table>

References


Modulation of T cell subset activation in vivo by CLIP


