DISCUSSION
The present study is an attempt to delineate the pathway of processing of exogenous antigen for presentation by MHC class I molecules. The system chosen for studying the generation of MHC class I-peptide ligand from exogenous antigen, is a T cell transfectoma which is MHC class I-restricted and ovalbumin-specific. B3.4.5 recognises the ovalbumin-derived peptide, SIINFEKL, bound to H-2K\textsuperscript{b}. Generation of MHC class I-peptide ligand was analysed following uptake of antigen through various routes—by pinocytosis, phagocytosis or receptor-mediated endocytosis. Antigen presenting capabilities of naive and activated T cells have also been explored. A transgenic murine model has been set up to address further the issues related to delivery of complete signal for responder T cell activation by naive T cells acting as APCs.

1. Presentation of exogenous antigen in association with MHC class I.

MHC class I molecules normally display peptides derived from intracellular proteins. MHC class I molecules are generally involved in presenting peptides to cytotoxic T lymphocytes, whose effector function is to eliminate actively infected cells. Hence, it is believed that MHC class I molecules do not acquire peptides derived from proteins in the extracellular milieu to prevent healthy cells from becoming sensitised for CTL lysis by foreign, noninfectious proteins released from neighboring infected cells. For generation of response from exogenous proteins, it has been shown that the protein should either be deliberately introduced in the cytosol of the cell [Moore et al, 1989], or administered in fusogenic form so that it is delivered to the cytosol.

However, there have always been anomalous reports of exogenous protein generating CD8 T cell responses that are MHC class I-restricted [Bevan et al,
1976; Huang et al, 1994], and over recent years these have crystallised into a controversial possibility that some macrophages can process exogenous antigen to present it on MHC class I [Rock et al, 1990]. Thus, before dissecting the processing pathway followed by exogenous antigen for presentation of its peptides in association with MHC class I, it was important to establish the occurrence of this phenomenon in the particular system used here. It has been suggested that some cells, mainly macrophages, can present antigens taken up by phagocytosis to MHC class I-restricted T cell lines [Kovacsovics-Bankowski et al, 1993]. However, phagocytic non-professional APCs have also been shown to present exogenous antigens [Reise Sousa and Germain, 1995]. Dendritic cells have also been shown to exhibit similar phenomenon although purified dendritic cells are non-phagocytic in vitro [Rock et al, 1992].

1.1 Macrophage cell line presents exogenous antigen on MHC class I.

In order to ascertain the cell type that would present exogenous antigen on MHC class I, a macrophage cell line and a B cell line were used as APCs. Although both the cell lines are capable of processing the antigen when it is present in the cytosol, through the classical MHC class I presentation pathway [Fig 1b], only the macrophage cell line is capable of efficiently stimulating MHC class I-restricted T cell line when antigen is given exogenously, whereas the B cell line cannot [Fig 1a]. It has been suggested that macrophages are highly phagocytic in nature, and hence are more efficient in taking up large amounts of antigen inside the cell. It is possible that increased concentration of exogenous antigen in the processing compartment allows its presentation on MHC class I in macrophages alone and since B cells are not so efficient in non-specific capture of antigen, there is no presentation on MHC class I.
It has also been suggested that particulate antigen will be presented far better by the macrophages than soluble exogenous antigen, again because phagocytosis is more efficient way of taking up the antigen as compared to pinocytosis. To test this possibility, OA was adsorbed on alum to make it particulate. Macrophage cell line presents particulate OA much better than soluble OA [Fig 2]. Some tumor cell lines have been reported to become phagocytic. So it was possible that transformed bone marrow-derived macrophages are highly phagocytic and hence, uptake of antigen is very efficient in them. It was also formally possible that the ability to present peptides derived from exogenous antigen on MHC class I is restricted to tumor cell lines. To test the occurrence of this phenomenon in ex vivo cells as well, macrophages purified from thioglycollate-primed peritoneal exudate cells [PECs] were used as APCs. Ex vivo macrophages also present particulate antigen more efficiently than soluble antigen [Fig 3]. However, it is likely that thioglycollate activates macrophages and increases the phagocytic activity of the macrophages to some degree. Since it is difficult to obtain relatively pure macrophage population from spleen cells or enough number of cells from unprimed peritoneal cells, thioglycollate-primed PECs remain the best source of relatively naive, ex vivo macrophages.

1.2 Antigen uptake by receptor-mediated endocytosis can also present peptides in association with MHC class I molecules.

Presentation of exogenous antigen in particulate form markedly increases the efficiency of MHC class I presentation by macrophages, but it is not clear whether phagocytosis merely augments antigen uptake, or has a qualitative effect on antigen handling. If uptake of the exogenous antigen contributes significantly to the presentation of peptide on MHC class I, then increasing
the uptake of the antigen should further enhance the presentation of exogenous antigen on MHC class I. Receptor-mediated endocytosis is known to be more efficient in antigen uptake than phagocytosis. Hence, OA was targeted to scavenger receptors present on the surface of macrophages. These receptors are expressed constitutively by macrophages and binds to polyanionic ligands [Krieger, 1992]. Maleylation is one of the chemical modifications which converts proteins into scavenger receptor ligand. It acylates both α- and ε-amino groups of the protein and confers negative charge [Haberland and Fogelman, 1985]. The scavenger receptor internalises the bound ligand very rapidly and efficiently through coated pits and in the lysosome, ligand is released from the receptor. Scavenger receptor then recycles back to the surface [Krieger et al, 1993].

Maleyl-proteins targeted to scavenger receptor on macrophages have been shown to be presented better to MHC class II-restricted T cells [Abraham et al, 1995], probably due to improved antigen uptake as a result of targeting. Maleyl-OA was used as exogenous antigen for presentation of OA to a MHC class I-restricted T cell line by macrophages. Despite increased uptake of maleyl-OA by macrophages as compared to OA, the MHC class I-peptide ligand generation is not different between maleyl-OA or OA [Fig 4] when processed and presented by macrophage cell line. This clearly demonstrates that increased concentration of antigen inside the cell does not account for the presentation of exogenous antigens on MHC class I. It also shows that phagosomes are not the only specialised compartments involved in processing of exogenous antigen for its presentation on MHC class I. Macrophages seem to have the unique capability to present exogenous antigen on MHC class I, no matter how the antigen is taken up in the cell.
Ex vivo peritoneal macrophages also generate equivalent response from MHC class I-restricted T cell line when either OA or maleyl-OA was used as exogenous antigen [Fig 6].

1.3 Maleyl-OA is presented very poorly when introduced in the cytosol.

Introduction of proteins in the cytosol of the cell, by osmotic lysis of pinosomes containing protein, allows processing of the protein in the cytosol by proteasome complexes in the cytosol [Moore et al, 1988]. Peptides generated are then transported by TAP across the ER membrane and in the lumen, they associate with newly formed MHC class I molecules. This method allows generation of MHC class I-peptide ligands from external protein. In order to ascertain whether processing of OA and maleyl-OA is equivalent in the cytosol, both the proteins were introduced in the cytosol of macrophage cell line. Although exogenous maleyl-OA does generate MHC class I-peptide ligands, surprisingly, maleyl-OA is presented very poorly when introduced in the cytosol of the cell [Fig 5]. Similarly, ex vivo peritoneal macrophages do not present cytosolic maleyl-OA very well [Fig 7], although both the macrophage cell line and peritoneal macrophages can process and present cytosolic OA efficiently on MHC class I [Fig 5 and 7]. This suggests that processing of an antigen is not similar when taken up through different routes into the cell.

The MHC class I-restricted ovalbumin-specific T cell line used here recognises an octapeptide derived from OA bound to H-2Kb. The sequence of the octapeptide is SIINFEKL. Alanine substitution analysis has shown that positions 4, 6 and 7 of amino acids in the peptide are important for recognition by cytotoxic T lymphocytes specific for OA [Jameson and Bevan, 1986].
Recent crystal structure analysis also shows that these positions are putative contact sites for the TCR and their side chains project out in the solvent [Fremont et al, 1995]. Positions 1, 3 and 8 contact residues lining the peptide-binding groove of MHC class I molecule. N-formyl modification of this OA peptide has been shown to inhibit binding of the peptide to MHC class I [Shawar et al, 1993]. The amino and carboxyl groups of the peptide are anchored in the peptide-binding groove and hence the length of peptide which can bind to MHC class I is restricted to 8-9 amino acids long. Replacement of the lysine residue at position 7, which has been shown to be important for TCR binding, with other amino acids, inhibits T cell response but does not affect binding of the peptide to MHC class I [Jameson et al, 1993]. Maleylation modifies both the α- and ε-amino groups. Since the octapeptide derived from OA contains a lysine residue, involved in recognition by the TCR, it is possible that processing of maleyl-OA in the cytosol generates a peptide in which Lys7 is maleylated. Hence, this peptide may not be recognised by the T cell line, although it may be loaded onto MHC class I molecules. To check this possibility, the synthetic octapeptide was maleylated and tested for recognition by the T cell line [Fig 8]. Maleyl-peptide is recognised very poorly by the T cell line, B3.4.5, although native peptide generates good response. However, it is possible that α-amino group of the synthetic peptide, being available, is modified by maleyl group and since substitutions at α-position have been shown to inhibit binding to MHC class I, maleyl-peptide may not bind to MHC class I at all. To determine whether nonresponsiveness to maleyl-peptide is due to non-binding of the peptide or non-recognition by the TCR, maleyl-peptide was used for MHC class I stabilisation on the TAP1/TAP2-deficient mutant cell line, RMA-S. Fig 9 shows that maleyl-SIINFEKL does not bind to MHC class I as well as native SIINFEKL peptide does.
To determine whether α-amino or lysine modification with maleyl group affects binding of the peptide to MHC class I, another H-2K\textsuperscript{b}-binding peptide, derived from VSV nucleoprotein, wasmaleylated and its capability to bind to MHC class I was tested. This peptide does not contain any lysine residue and the only site available for maleylation is the α-amino group. Since amino group has been clearly demonstrated to play an important role in anchoring the peptide to MHC class I peptide-binding groove, it was expected that maleyl-VSV peptide will not bind to MHC class I. However, it is found that both the native and maleyl-VSV peptide are equally efficient in restoring the expression of MHC class I on the mutant cell line, RMA-S [Fig 10]. This suggests that modification of α-amino group with maleyl residue does not affect binding and the decreased binding of maleyl-SIINFEKL peptide may be due to modification of lysine residue. Since this is contrary to the predictions of the crystallographic data [Fremont\textit{ et al}, 1995], it was necessary to address the role of lysine in binding of the peptide to MHC class I.

In this context, lysine was substituted with glutamic acid [SIINFEEL]. This substitution has been reported to affect TCR recognition, but not binding to MHC class I [Jameson\textit{ et al}, 1993]. The ability of glutamic acid-substituted peptide to stimulate T cell line was checked and Fig 11 shows that substituted peptide is not recognised by the T cell line, demonstrating the importance of lysine in recognition by TCR. Substitution of lysine with glutamic acid permits binding to MHC class I molecules [Fig 12]. Maleylation of this peptide would attach maleyl groups at only the α-amino position as there is no lysine present and if indeed abrogation of binding of the peptide to MHC class I is not due to modification of lysine but of α-amino group, maleyl-SIINFEEL would not bind. However, maleyl-SIINFEEL does bind to MHC class I quite well, whereas maleyl-SIINFEKL does not [Fig 12]. This again suggests that
Discussion

lysine residue, till now thought to be involved only in TCR binding, does
play a role in binding of the peptide to MHC class I also. However, it is
possible that the loss of binding of maleyl-SIINFEKL is due to some unique
property of maleyl group and other substitutions at this position may not
affect binding. It has been reported that attachment of fluorescein molecules
at lysine permits binding of the peptide to MHC class I [Day et al, 1995]. To test
the above possibility, peptides were labeled with biotin since biotin also
attaches to both α- and ε-amino groups through isopeptide bond. Only
SIINFEEL-biotin binds to MHC class I [Fig 13], and stabilises MHC class I on
the surface of mutant cell line, RMA-S [Fig 14b]. SIINFEKL-biotin does not
bind to MHC class I [Fig 14a], demonstrating the role played by a TCR-
contacting residue in binding to MHC class I. The modification of lysine may
either directly hinder binding of the peptide or maleyl or biotin chains may
sterically hinder the binding of neighboring residues, which have been
implicated in anchoring the peptide to MHC class I peptide-binding groove.
Exact mode of inhibition of binding of peptide by these modifications would
need detailed crystallographic analysis. However, the inability of Lys7 maleyl-
SIINFEKL to bind to MHC class I is quite possibly the reason why maleyl-OA
cannot be presented cytosolically to generate the ligand.

Endogenous proteins are degraded in the cytosol through proteasomal
complexes-[Goldberg-and Rock, 1992; Rock et al, 1994]. Proteins are first
conjugated to ubiquitin molecules through ε-amino groups of the protein
and C-terminal glycine of the ubiquitin molecules [Hershko and
Ciechanover, 1992]. Multiple ubiquitin molecules are attached to the protein.
This is recognised by the 26S proteasome subunit and the protein is then
degraded into peptides [Goldberg and Rock, 1992]. Generation of peptides
through degradation by proteasomal complex is the main pathway in
presentation of endogenous protein by MHC class I [Michalek et al, 1993]. In maleyl-OA, since ε-amino groups are already blocked by maleyl groups, it is possible that ubiquitin conjugation does not take place. In absence of ubiquitin, protein will not be degraded by proteasomes. This could be another likely explanation for reduced response from cytosolic maleyl-OA.

Presence of maleyl group on the protein leads to conformational changes. It increases negative charge on the protein and also modifies the 3-dimensional structure of the protein. Maleylation has also been shown to generate new epitopes, both for B cells and for T cells, in the protein [Abraham et al, 1995]. Hence, it is possible that because of the presence of maleyl groups, processing of maleyl-OA is altered in the cytosol and completely new set of peptides are generated from maleyl-OA. It has been shown that treatment of maleylated proteins at acidic pH removes the maleyl groups from lysine residues. If the conformation of protein was lost during maleylation leading to poor response from cytosolic maleyl-OA, then demaleylation of maleyl-OA will not restore the presentation of peptide on MHC class I. However, demaleylated maleyl-OA generates a response when introduced in the cytosol of macrophages [Fig 15]. Thus, it appears that the maleyl residues are directly involved in the non-recognition of cytosolic maleyl-OA by B3.4.5.

1.4 Processing of exogenous maleyl-OA is more sensitive to change in pH of the endosomes.

Exogenous maleyl-OA generates relevant MHC class I-peptide complexes whereas cytosolic maleyl-OA does not. It seems that processing of the same antigen is different in different processing compartments. From maleyl-peptide binding data, it is clear that for generation of the right peptide from
maleyl-OA, removal of maleyl group is necessary, either from the protein before processing or from the peptide after it is generated. Exogenous antigens are taken up in the endosomes where acid proteases degrade the antigen into peptides. Low pH in the endosomes enhance the activity of proteases and may also play a role in destabilising the structure of native protein, facilitating its degradation. It is possible that exogenous maleyl-OA taken up by receptor-mediated endocytosis go to endosomes and in the presence of low pH, get demaleylated either before processing or after processing. This would lead to generation of relevant peptide which is presented in association with MHC class I molecules. Processing of cytosolic protein takes place at physiological pH and since demaleylation cannot occur at this pH, cytosolic maleyl-OA does not generate a response. If processing of exogenous maleyl-OA is dependent on low pH of the endosomes, then change in endosomal pH should decrease the response. Ammonium chloride is a weak base and increases endosomal pH. It has been shown to inhibit endosomal proteases, thereby reducing the presentation of exogenous antigen on MHC class II. To a certain extent, ammonium chloride affects presentation of exogenous antigen on MHC class I also, but only at higher concentrations [Michalek, et al, 1991].

In presence of ammonium chloride, presentation of exogenous OA is not affected at all, at the concentrations used. However, exogenous maleyl-OA presentation is completely inhibited even by very low concentrations of ammonium chloride [Fig 17], suggesting that acidification of maleyl-OA in the endosomes is required probably for demaleylation of the antigen before relevant peptide can be generated from it. It is likely that demaleylation is not very efficient and hence, despite increased uptake of maleyl-OA by the
Discussion

macrophages, as compared to OA, the response generated by the two antigens is equivalent.

1.5 Processing of exogenous OA and maleyl-OA does not take place in the cytosol.

The processing pathway for exogenous antigen for presentation on MHC class I, is not very well understood till now. Some exogenous proteins primed CTL responses \textit{in vivo}, and this was attributed to the unique ability of these antigens to be delivered into the cytosol. Such antigens included some bacteria such as those that disrupt membranes and escape to the cytosol, fusogenic viruses that penetrate membranes, and, of course, peptides that can bind directly to MHC class I on the surface and do not require further processing. However, generation of CTLs specific for allogenic peptides presented on self MHC class I molecules, after priming with allogenic transplants that lack appropriate MHC class I for presenting peptides to T cells [Bevan, 1976], cannot be explained by entry of alloantigen into the cytosol. Antigens associated with cell debris [Debrick \textit{et al}, 1991], or encapsulated in liposomes [Reddy \textit{et al}, 1992], or antigens denatured by boiling or SDS treatment [Martinez-Kinader \textit{et al}, 1995] do not have any direct mode of entry to the cytosol, yet they generate MHC class I-restricted responses.

Three different pathways have been described as contributing to exogenous antigen presentation by MHC class I molecules. According to some reports, exogenous antigens are transferred into the cytosol due to the leaky nature of phagosomes [Kovacsovics-Bankowski and Rock, 1995]. This has also been used as an argument to prove that phagosomes are the only specialised compartments involved in the presentation of exogenous antigen by MHC
class I. However, data presented here show that exogenous antigen taken up in the endosomes can also access MHC class I. However, it is possible that such endosomes also leak some amount of proteins into the cytosol, and the presentation of such exogenous antigen would then be through the classical MHC class I processing pathway. For presentation of exogenous maleyl-OA, it is possible that maleyl-OA is demaleylated in the endosomes and then are transferred to the cytosol where it is degraded by proteasomal complexes. If this pathway is operative then it would require the presence of newly formed MHC class I molecules and MHC class I-peptide complexes would move out of the ER, through Golgi complex and on to the surface of the cell. This presentation would be blocked by brefeldin A, a drug that inhibits egress of proteins from ER. Fig 18b shows that presentation of neither exogenous OA nor maleyl-OA is affected by brefeldin A, clearly showing that exogenous native antigens do not enter cytosol.

It is still possible that exogenous antigens are processed in the endosomes and the peptides are transferred to the cytosol, since MHC class I molecules are not known to be transported via endosomal compartments, unlike MHC class II molecules which are targeted to endosomes by Ii chain. Hence, peptides transferred to the cytosol are transported by the TAP molecules into ER lumen where they can associate with MHC class I molecules. Both TAP-dependent [Kovacsovics-Bankowski and Rock, 1995; Reise Sousa and Germain, 1995; Huang et al, 1996] and TAP-independent pathways [Harding and Song, 1994; Pfeifer et al, 1993; Bachmann et al, 1995] have been reported for the presentation of exogenous antigen. To check whether TAP is required for presentation of exogenous antigen taken up by pinocytosis or receptor-mediated endocytosis in the present system, peritoneal macrophages from mice lacking TAP1 were used to present OA or maleyl-OA to MHC class I-
Discussion

restricted T cell line. TAP-deficient macrophages can present OA and maleyl-OA as efficiently as normal macrophages [Fig 20], suggesting that these proteins are presented in a TAP-independent manner. This suggests that neither protein nor peptides are transferred to the cytosol for presentation on MHC class I. It has been suggested that exogenous proteins are degraded in the endosomal compartments and peptides are regurgitated outside the cell where they associate with surface MHC class I [Pfeifer et al, 1993; Harding and Song, 1994]. It is possible that peptides are regurgitated and associate with empty MHC class I molecules on the surface of TAP1-deficient macrophages in the present system as well. However, another pathway has been recently reported which is TAP-independent and does not involve peptide regurgitation [Bachmann et al, 1995]. MIIC compartment, a specialised endosomal compartment for loading of peptide on MHC class II has recently been reported [Amigorena et al, 1994; Peters et al, 1991; Tulp et al, 1994; Rudensky et al, 1994]. It is speculated that this compartment may be responsible for TAP-independent loading of MHC class I molecules by macrophages.

The data clearly suggest that CTL responses can be generated from exogenous proteins and there is probably an interconnection between MHC class I and MHC class II pathways. However, MHC class I presentation of exogenous antigen is less efficient compared to presentation of endogenous proteins. Higher concentrations of exogenous proteins are required to generate MHC class I-peptide complexes. Although relatively inefficient, this pathway may explain generation of CTL responses against bacteria that replicate in the endosomes. This processing pathway may be advantageous for the immune system. It may allow induction of CTL response against tissue-specific viruses that infect other somatic cells but avoid professional APCs. Partial violation
of the principle of MHC class discrimination by professional APCs may allow induction of efficient CTL responses without lysis of innocent bystander cells. Parasites like Mycobacterium and Leishmania can survive and replicate in damaged macrophages. Exogenous pathway for MHC class I loading may allow destruction of such chronically infected cells.

2. Antigen presentation by T cells.

T cells can present antigens under certain circumstances. T cells have very high expression of MHC class I molecules and upon activation with mitogens or antigens, human T cells express MHC class II molecules. Activated T cells express CD80 or the adhesion molecule, B7. This molecule has been implicated as an important co-stimulatory factor. Various reports show that T cells can present antigenic peptides to responder T cell clones [LaSalle et al, 1991]. In other reports, presentation of peptides by T cells were found to induce anergy [Sidhu et al, 1992]. Induction of anergy is thought to occur due to poor secondary signals provided by T cells. However, it has been shown that despite the expression of B7 molecule, there is induction of anergy [Satyaraj et al, 1995; Lombardi et al, 1994]. T cells cannot present peptides from intact proteins on MHC class I, which is thought to be due to inability of T cells to capture and process antigens efficiently. However, targeting antigens to CD4 co-receptors enhance uptake of antigen by T cells and leads to presentation of antigen [Lanzavecchia et al, 1988].

It has been previously demonstrated that activated T cells and some virus-infected cells spontaneously endocytose and recycle their MHC class I [Tse and Pernis, 1984]. The process has been shown to involve internalisation of MHC class I molecules into acidic endosomal compartments [Hochman et al, 1985]...
Fibroblasts and LPS-activated B cells do not exhibit internalisation of MHC class I [Tse and Pernis, 1984]. It has been speculated that during recycling of MHC class I molecules through endosomal compartments, bound peptide is either dissociated or exchanged with other peptides present in endosomes, which normally load on MHC class II molecules, but no data are so far available.

To address the issue of processing of endogenous proteins and the generation of proper MHC class I-peptide ligand in T cells, *ex vivo* T cells were loaded with OA by osmotic lysis of pinosomes. Generation of MHC class I-peptide was compared with professional APCs, namely B cells. Fig 21a shows that T cells can process endogenous proteins and present peptides on MHC class I as efficiently as professional APCs, such as B cells. The presentation of synthetic peptide is marginally better than with other APCs [Fig 21b], probably since T cells have higher expression of MHC class I molecules on the surface.

To test whether activation of T cells does lead to a change in peptide repertoire presented by MHC class I, con A-activated T cells were loaded with OA by osmotic lysis of pinosomes. The response generated by the synthetic peptide was lower when it was presented by activated T cells as versus activated B cells or naive T cells [Fig22]. This suggests that during recycling of MHC class I through endosomes, peptide is lost or exchanged in the endosomes. Treatment of cells at 4°C stops membrane recycling. To ascertain that the peptide is lost after internalisation of MHC class I-peptide complex, and not dissociated on the surface, activated T cells were incubated with peptide at 4°C. This blocks membrane recycling by making the lipid bilayer of the membrane rigid. Internalisation of MHC class I cannot occur in this case. If the peptide is lost inside the cell, after internalisation, then treatment at
4°C should restore the response. This is shown in Fig 23, where treatment of naive and activated B cells and naive T cells at lower temperature does not make any difference to peptide responses, whereas, treatment of activated T cells at lower temperatures enhances the peptide response, clearly suggesting that peptide dissociates from MHC class I after it is taken inside the cell and either empty MHC class I or MHC class I bound to new peptides recycle back to cell surface.

The function of MHC class I internalisation in activated T cells is still a matter of speculation. One interesting possibility is that recycling of MHC class I allows alteration in associations with other integral membrane proteins or antigenic peptides. Activated T cells recycle through endosomes where pH is 5.6. At this pH, β2-m dissociates from MHC class I and may reassoclate with MHC class I heavy chain during transit back to the cell surface. This reversible dissociation may help to increase conformational flexibility of the heavy chain and induce a transitory opening of the peptide binding groove. This would facilitate exchange of peptides in endosomal compartments and allow generation of CTL responses against proteins normally taken up in the endosomes which do not reach cytosol. Why T cells possess this unique ability, and what role it plays in infection, are yet open question.

3. Development of an experimental system with an IgH transgenic mouse line to analyse transgenic protein presentation.

For complete activation of T cells two signals are required. The first signal is constituted by specific MHC-peptide complex and is antigen specific. The second signal is mediated by certain co-stimulatory molecules present on the
surface of APCs and is thought to be non-specific. Deliverance of both the signals is necessary for proper T cell activation. T cells cannot be activated by co-stimulation in the absence of first signal and engagement of TCR with specific MHC-peptide ligand without proper co-stimulation leads to induction of anergy [Jenkins et al, 1988]. Although T cells have been demonstrated to express specific MHC-peptide ligand and are capable of delivering the first signal for responder T cell activation, it is not clear whether proper co-stimulus can be provided by T cells. Most of the work involving antigen presentation by T cells use antigen-specific T cell clones. Co-stimulatory requirements for priming of naive T cell are more stringent than for generating a memory response [Dubey et al, 1995].

To address the issue of priming of antigen-specific T cells by stimulator T cells, characterisation of an IgH transgenic mouse line has been initiated. Transgene is expressed under the control of μ-enhancer element which is utilised in both T and B cells. In T cells the transgenic protein is expected to remain intracellular in the absence of light chain whereas in the B cells transgenic protein is expressed on the surface. Since transgenic protein would be found in both B and T cells, it would be easier to compare processing of this endogenous protein in professional APC versus non-professional APC. Before starting analysis of generation of MHC class I-peptide ligand in T and B cells, it is necessary to confirm the presence of transgenic protein in T cells. Almost all the peripheral lymphocytes from transgenic animals show presence of intracellular transgenic protein [Fig 24] suggesting that B cells as well as T cells express transgenic protein inside the cell. Stainings of the spleen cells from transgenic animals show that only B cells [B220⁺] express the transgenic protein on the surface [Fig 25b]. However, post-permeabilisation stainings show that in addition to B cells, B220⁻ [T] cells in
transgenic animals also express transgenic protein intracellularly and the expression is similar to that in the B cells [Fig 25d].

Monoclonal antibodies were generated against the VDJ region of the transgenic heavy chain [Fig 27 and 28] for further analysis of transgenic protein expression. To address the issue of generation of MHC class I-peptide from endogenous transgenic protein in T cells, CD8+ T cell hybridomas specific for transgenic protein were generated. These T cell hybridoma recognise transgenic spleen cells, specifically, but not non-transgenic spleen cells [Fig 29]. These hybridomas are currently being re-established for stability, and can then be used to study processing of marker endogenous protein in T cells versus B cells.