SUMMARY AND CONCLUSIONS
This study has attempted to describe processing pathway followed by exogenous antigen for presentation by MHC class I molecules.

1. Macrophages present exogenous antigen on MHC class I.

Macrophage cell line and peritoneal macrophages present exogenous OA much better than B cell line. Presentation of phagocytic OA is better than presentation of fluid-phase OA, possibly due to higher uptake of antigen through phagocytosis. Targeting OA to scavenger receptors present on macrophages, after maleylating OA, leads to excellent presentation on MHC class I, ruling out the possibility that phagosomes are the only specialised compartments capable of loading exogenous antigens on MHC class I.

2. Maleyl-OA, when present in the cytosol, is not presented on MHC class I.

Processing of maleyl-OA in the cytosol, through cytosolic proteasome complexes, does not generate relevant MHC class I-peptide complex. This could be either due to generation of a different peptide in which lysine residue is modified with maleyl group, or due to non-degradation of maleyl-OA in absence of ubiquitin conjugation. Synthetic maleyl-peptide does not bind to MHC class I and this is due to modification of lysine residue, which has till now been implicated in only binding to TCR. Addition of maleyl group to amino terminal does not affect binding of the peptide to MHC class I. Inhibition of binding of the peptide by the presence of maleyl group is not specific property of maleyl group, since labelling peptide with biotin also abrogates binding of the peptide to MHC class I. Biotin at amino terminal of the peptide does not affect binding. The effect of maleyl substitution at Lys7 on the peptide could be either due to direct inhibition of binding of the
residue to MHC class I groove, or more likely, due to steric hinderance in binding of neighboring residues, which have been shown to be important in anchoring the peptide in MHC class I groove.


Generation of response from exogenous maleyl-OA suggests that native, non-maleylated peptide is generated from its processing, which binds to MHC class I. For this, it is essential that maleyl-OA should be demaleylated, either before processing or after the generation of peptide. Antigens taken up by receptor-mediated endocytosis enter endosomes and lysosomes and in presence of low pH and acid proteases present there, antigens are degraded into peptides. It is, therefore, likely that maleyl groups are removed from exogenous maleyl-OA in these compartments and native peptide is generated. This is supported by the fact that presentation of maleyl-OA is drastically reduced by low concentrations of ammonium chloride, a weak base that increases pH of the endosomes. Presentation of exogenous OA is not affected by ammonium chloride, suggesting that the two antigens are either processed differently or are processed in different compartments of the cell.

4. Presentation of exogenous antigens occurs through TAP-independent pathway.

MHC class I molecules are not, normally, transported through the endosomal compartments. Hence, it is unclear how peptides generated from exogenous antigens reach MHC class I molecules. One of the possibilities is that antigens are transported into the cytosol, either through an active
transport mechanism operative in phagosomes/lysosomes or through non-specific leakage from these compartments. The antigen is then processed in the cytosol and transported to ER lumen where they associate with newly synthesised MHC class I molecules. However, this pathway does not seem to be operative as presentation of both exogenous OA and maleyl-OA is unaffected by brefeldin A, which inhibits egress of newly synthesised molecules from ER. Moreover, peritoneal macrophages taken from TAP1-deficient mice do present exogenous OA and maleyl-OA very well on MHC class I. This suggests that exogenous antigens are not processed in the cytosol since presentation of peptide derived from OA does not require transport by TAP. It is possible that OA and maleyl-OA are processed in the endosomal compartments and peptides generated are regurgitated into the extra-cellular milieu where they associate with MHC class I molecules present on the surface of the cell, or that this binding occurs in the presence of a unique compartment, where MHC class I molecules interact with peptides derived from exogenous antigens.

5. Activated T cells present peptide very poorly.

T cells express high levels of MHC class I and hence, can be expected to process and present endogenous proteins in association with MHC class I. T cells present OA when it is introduced in the cytosol by osmotic lysis of pinosomes. Presentation of peptide is marginally better than other professional APCs, probably because of higher levels of MHC class I molecules on T cell surface. Activated T cells internalise and recycle MHC class I molecules very rapidly through endosome-like compartment. Presentation of peptide by activated T cells is poor as compared to naive T cells or activated B cells. It is possible that peptide is lost in the acidic
endosomal environment. Blockade of membrane recycling, by peptide loading of cells at 4°C, enhances the peptide response by activated T cells, whereas responses of other cells are not affected. This shows that bound peptide is exchanged during recycling of MHC class I molecules through acidic compartments, probably endosomes.

6. Development of IgH transgenic system to analyse issues related to antigen presentation by T cells.

The characterisation of an IgH transgenic system has been begun to address issues related to antigen processing and presentation by T cells, and the consequences of such T-T interactions. The transgenic Ig heavy chain is expressed in both T cells and B cells. In B cells, it is expressed both intracellularly and on the surface, in association with endogenous light chain, whereas in T cells, it remains largely intracellular, in absence of light chain. The levels of expression of transgenic IgH is similar in both T and B cells. Monoclonal antibodies have been generated against VDJ region of the transgenic heavy chain. T cell hybridomas can be generated against transgenic protein for use to study the expression of MHC class I-peptide complex from processing of transgenic protein in both T cells and B cells.