SUMMARY AND CONCLUSION

In this study, we have attempted to characterise antigen presentation and accessory signaling, predominantly with non-transformed human T cells. The study was carried out using the allorecognition system, with freshly isolated T cells as responders, and short-term activated [48 h] T cells as APCs for a T-T interaction.

The first question that we addressed was, whether activated T cell APCs expressing MHC class II allo-ligands can elicit a primary alloresponse from freshly isolated CD4 T cells, and the answer was in the negative. When responder-syngeneic 'by-stander' cells were added, in an attempt to see if provision of 'by-stander' help could overcome any lack of co-stimulation by the activated T cell APCs, a small but significant proliferative response was obtained to the activated T cell APCs. However, this turned out to be an artifact of the allorecognition system, because the 'by-stander' help seen was haplotype specific and, therefore, was not genuine 'by-stander' help. Freshly isolated responder T cells also failed to mount a primary alloresponse against a 15-day old T cell line, which has been shown to deliver activating signals to responder T cells, in other experimental systems. T cell APCs also failed to stimulate T cell proliferation from PBMC-primed responder T cells, which are expected to need less stringent stimuli, as compared to, the freshly isolated T cells for activation. It therefore appeared that, either the MHC class II allo-ligands on activated T cell APCs were being completely ignored by the responder T cells or that the activated T cell APCs were inducing tolerance in the responder T cells. We therefore decided to analyze the consequence of antigen presentation by activated

T cell APCs.

We have shown here that antigen presentation by activated T cell APCs induces ligand-specific tolerance in the responder T cells. Following T-T interaction, responder T cells are rendered non-responsive to the MHC class II allo-ligands expressed on the activated T cell APCs, while their responses to polyclonal T-cell stimulating agents, such as PHA and anti-CD3, as well as 'third party' allo-ligand is not affected. The expression of MHC class II allo-ligand is essential and mandatory for the induction of tolerance, because responder-syngeneic activated T cell APCs do not induce tolerance. Thus, the tolerance induced is not only ligand-specific, but is also not the result of some secreted suppresser factor. The induction of tolerance to MHC class II allo-ligands, presented by professional APCs, suggested that there is substantial cross-reactivity between professional APCs and T cells, with regard to the peptides bound to the MHC class II molecules.

Tolerance could be due to deletion of T cells or induction of anergy. We attempted to characterise the mechanism of induction of tolerance in our system. We have shown, that the tolerized T cells show higher response to IL-2, a characteristic associated with 'anergy' as the mechanism of tolerance. Also, in other systems anergy induction has been shown to be prevented by addition of exogenous IL-2 and is true in our system, as well, indicating that the availability of IL-2 may be one of the a crucial factor in the induction of anergy. Further investigation into the mechanism of induction of anergy showed that simple deprivation of

available IL-2, by addition of 'by-standers' T cells expressing high-affinity IL-2R, does not lead to the induction of anergy during priming with professional APCs, like PBMCs.

Further, the induction of tolerance is independent of the co-receptor molecule expressed on the activated T cells. Both CD4 and CD8 T activated T cell APCs induce tolerance in responder T cells. This ruled out the possibility of CD8 co-receptor molecule specifically inducing tolerance, possibly by killing the responder T cells.

Of the co-stimulatory molecules that responder T cells need for being activated rather than inactivated, the interaction between CD28/CTLA-4 on the responder T cell and B7 family of ligands on the APCs appears to be crucial. Analysis of CD28/CTLA-4-B7 interaction in our system showed that 48-h activated T cell APCs express CTLA-4-binding ligands. There are reports of the expression of B7 family of ligands on T cells that have been activated for, at least, a week or more and in that context our finding is unusual. Despite CTLA-4-binding ligands being expressed on activated T cell APCs, they were unable to elicit an alloresponse and they induced anergy. This suggested that, either these ligands were not functionally available for interacting with the CD28/CTLA-4 molecules on the responder T cells, or that CD28-B7 interactions may not be relevant in T-T interaction.

When we provided CD28 mediated co-stimulation, using anti-CD28 antibody, the activated T cell APCs were still unable to elicit a

primary alloresponse from the responder T cells. Further, addition of CD28 antibody during priming with activated T cell APCs did not prevent the induction of anergy. This suggested that CD28-B7 interaction may not be relevant in T-T interaction of the kind that we have studied.

One other possibility to explain the anergy induced is that the activated T cell APCs may be giving a 'negative' signal to the responder T cells. We have tried to see if paraformaldehyde fixation of the activated T cells, can prevent the induction of anergy. Preliminary data suggests, that even paraformaldehyde fixed activated T cell APCs can induce anergy. A T-T interaction is bi-directional, in the sense, that both the stimulators and the responder express molecules such as CD28, LFA-3, CD3. ICAM-1 & ICAM-3, *etc.* This could be the reason for the outcome. Besides the activated T cell APCs express CTLA-4 on their surface, and it has been shown that CTLA-4 molecule on the APCs could lead to down modulation of the immune response. However, the precise molecular mechanisms resulting in the induction of anergy following T-T interaction are still far from clear.