SUMMARY

It is a widely held opinion that for optimal activation of T cells, two distinct signals are required. The first signal is generally provided by the crosslinking of the T cell receptor (TCR) with antigen-MHC complex, while the second signal also known as the costimultory signal, is provided by the antigen presenting cell (APC), as a result of its cognate interaction with the T cell. It is also reckoned that in the absence of the costimulatory signal, the T cells become tolerized. Literature does not record concrete evidence regarding the nature and the *modus vivendi* of these costomulatory signals. Though several reports have come up specifying different molecules to have costimulatory activity, the inventory of such signals is still incomplete. Given this background, the dissection of costimulatory signals endowed by the macrophages, was attempted in our lab.

Since the study was aimed and designed to identify costimulatory molecules from macrophages, plasma membrane from mouse peritoneal macrophages was isolated. The membrane bound proteins were separated out from the solubilized membrane extract by SDS-PAGE and reconstituted individually into lipid vesicles, for restoration of their native conformations. Each protein was tested for its ability to provide costimulatory signal to naive, resting T helper cells, after crosslinking the TCR by anti-receptor antibodies, as the first signal. A 148-155 kDa protein band, which we named M150, provided costimulatory signal to the T helper cells in our system, leading to their proliferation and differentiation. Two-dimensional gel electrophoresis of this band verified it as comprising of a single protein only.

Utilizing the compulsive and credible technique of electron-microscopic autoradiography, we were able to demonstrate the binding of radiolabeled M150 to anti-CD3 activated T cells, as only the T cells which have received the first signal, seem to express the receptors for this molecule. The binding of M150 was thought to be the prerequisite for costimulation, for it triggered off a whole cascade of biochemical events. The earliest of these was the phosphorylation, at tyrosine residue, of a 32 kDa protein, which was detected by an anti-phosphotyrosine antibody. It was closely followed by the activation of protein kinase C (PKC), because complete PKC translocation to the membrane resulted in M150 activated T cells, in our experiments. No increase in intracellular

calcium content revealed that the signal provided by M150 was independent of calcium mobilization event, which is a distinct property of a costimulatory signal.

The ultimate outcome of all the biochemical events was the transcription of lymphokine genes. Qualitative analysis of total, T cell mRNA, by Northern blotting, indicated the presence of mRNAs for IL-2 and IFN-γ. The lymphokines secreted, were also tested quantitatively, using the specific indicator cell lines. IL-2 and IFN-γ were the only lymphokines present in the culture supernatant of the M150 activated T cells, a characteristic feature of T_H1 type of cells. All the presently known costimulatory molecules, apart from providing costimulatory signal to T cells, are adhesive in nature. Our studies demonstrated that M150 was unable to cause adhesion of T cells to the culture plates indicating its non-adhesive nature.

The novelty of M150 as a costimulatory molecule has been amply discussed in the light of earlier reports about other costimulatory molecules. The overall results of the study lead to a postulation that, either M150 is a costimulator specific for T_H1 cells, or it commits the naive T cells to acquire T_H1 phenotype only. Our study demonstrates that M150 is a possible candidate for a position, in the inventory of costimulatory molecules.