SYNOPSIS

Synopsis of the work done by **Girdhari Lal** (SR No 111300302) for the award of Ph D degree in the faculty of Science, Indian Institute of Science, Bangalore, India

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Perpetuation of T cell memory: A role for anti-idiotypic T cells

Introduction

After infection or vaccination, many organisms can 'remember' the identity of the encountered pathogen (antigen), which helps them to respond with enhanced vigour and protect them from re-infection, sometimes for life The factors that maintain antigen specific memory are not clearly understood These include triggering of memory cells by self-antigen, cross-reactive environmental antigen or certain cytokines However, self-antigen triggering would lead to autoimmune disease and attrition The source of cross-reactive antigens is undefined, and cytokine stimulation is a generalized effect Furthermore, different duration of antigen specific memory for different antigens is not explained A hypothesis known as "relay hypothesis" has been proposed earlier (Nayak *et al*, Immunol 2001, 102 387), which provides a mechanism for maintenance of B cell memory through idiotypic interactions and selection involving idiotypic and anti-idiotypic B cells This hypothesis has proposed that T cell memory is a by-product of B cell memory as B cells present idiopeptides derived from complementary determining regions (CDRs) to T cells T cell memory co-perpetuates with B cell memory

Objectives of present investigation

The aim of the present investigation is to provide experimental evidences for the maintenance of T cell memory through interaction of idiotypic and anti-idiotypic T cells

The specific objectives of present studies are-

1 Extension of relay hypothesis for T cell memory and postulation of possible testable mechanisms for T cell memory

2 Providing experimental support for the generation of idiotypic T cells and anti-idiotypic T cells using a viral protein and ovalbumin

3 Experimental demonstration of T cell idiotypic network and generation of memory T cell response

The first chapter describes the proposed hypothesis According to this hypothesis, anti-idiotypic T cells are generated during the immune response due to processing and presentation of idiopeptides (peptide derived from the variable region of TCR) by idiotypic T cells or engulfment of apoptotic idiotypic T cells by phagocytic cells Anti-idiotypic T cells are generated in the body after processing and presentation of TCR from dead idiotypic T cells by phagocytic cells in the contraction phase of immune response Idiotypic T cells can process and present their TCR due to the degradation of down regulated surface TCRs to cognate anti-idiotypic $CD4^+$ T cells The DRiPs formed in the T cells also act as the source for idiopeptides in the T cells and generate anti-idiotypic $CD8^+$ T cells The TCR of anti-idiotypic T cells are postulated to carry antigen-mimic (peptidomimic) and act as surrogate antigen. Interaction of idiotypic and anti-idiotypic T cells directly or through idiopeptides presenting phagocytic cells leads to their proliferation followed by regulation of their population by cytotoxic T lymphocytes This investigation delineates the events involved in the generation, maintenance and regulation of T cell memory mediated by the network comprising of complementary idiotypic, anti-idiotypic T cells and phagocytic cells

Generation of idiotypic CD4+ T cell clone

Recombinant nucleocapsid protein (N protein) of Rinderpest virus (RPV) was expressed in *E coli* and purified Balb/c mice have been used as animal model for all experiments To study the role of idiotypic anti-idiotypic T cells, a $CD4^+$ T cell clone and $CD8^+$ T cell hybridoma specific for N protein have been generated The antigen specificity, MHC restriction, epitope mapping and V_β usage have been determined Chapter 2 describes generation and characterization of $CD4^+$ T cell clone

Processing and presentation of TCR by idiotypic T cells

The expression of MHC class II molecules on activated T cells has been demonstrated Activated mouse T cells are able to present extra-cellular viral proteins to cognate T cells Activated T cells down regulate their surface TCR, which is taken into lysosomal compartment as monitored by co-localization of surface biotinylated TCR with LAMP-1 using confocal microscopy These down regulated TCRs are processed by lysosomal enzymes and presented to cognate anti-idiotypic $CD4^+$ T cells in MHC II dependent manner as monitored by IL-2 secretion, *in vitro* proliferation and cell cycle progression from G_0/G_1 to G_2/M

Newly synthesized TCR proteins are formed as Defective Ribosomal Products (DRiPs), as shown by inhibition of degradation of [^{35}S]-methionine labeled cellular proteins as well as TCR protein in presence of lactacystin. These TCR-DRiPs have been shown to be processed by proteasome Presentation to cognate anti-idiotypic $CD8^+$ T cells through MHC class I molecules has been shown Presentation of idiopeptide was detected by cell adhesion between idiotypic and anti-idiotypic T cells, secretion of IL-2 and IFN-γ by anti-idiotypic $CD8^+$ T cells Presentation of idiopeptides by idiotypic T cells has been shown to up-regulate bcl-2 molecules in anti-idiotypic $CD8^+$ T cells as monitored by FACS. Chapter 3 describes the expression of MHC class II molecules, processing and presentation of surface T cell receptor as well as TCR DRiPs by activated idiotypic T cells to anti-idiotypic T cells

Generation of anti-idiotypic T cells, operation of T cell idiotypic network and memory T cell response using morbillivirus N protein.

In the present work, using adoptive transfer of idiotypic T cell clone in syngenic naive mice, generation of anti-idiotypic $CD4^+$ and $CD8^+$ T cells have been monitored by *in vitro* proliferation assay using [^{3}H]-thymidine incorporation, intracellular staining of IFN-γ by FACS after *in vitro* stimulation with apoptotic T cell clone pulsed dendritic cells as antigen presenting cells N protein exhibits Vβ3, Vβ8 1, Vβ13 repertoire dominant immune response Anti-idiotypic T cells have been generated after immunization with antigen enriched polyclonal idiotypic T cells Using lethal irradiation of N-primed animals and reconstitution of syngenic bone marrow cells intravenously, anti-idiotypic T cells have been generated Operation of idiotypic network for T cell has been monitored after injection of idiotypic $CD4^+$ T cell clone as detected by presence of idiotypic $CD8^+$ and anti-idiotypic $CD8^+$ T cells in the same animal Boosting of animals 72 days after antigen priming with anti-idiotypic T cells generates antigen specific $CD4^+$ and $CD8^+$ T cell memory response This experiment was performed using recombinant nucleocapsid protein as model antigen Chapter 4 describes this part of work

Generation of memory T cell response using Ovalbumin

In order to provide additional evidence for the relay of memory through complementary TCRs, ovalbumin has been used as antigen The TCR beta chain specific for ovalbumin was cloned and expressed in *E coli* Ovalbumin immunized mice boosted with recombinant protein or DNA generated anti-idiotypic T cells and antigen specific memory This work has been described in chapter 5