MOLECULAR ANALYSIS OF CYTOKINE POLYMORPHISM IN CONJUNCTION WITH THE STUDY OF IMMUNE RESPONSE TO COMMON RECALL ANTIGENS, SUPERANTIGENS AND XENOBIOTIC COMPOUNDS IN AUTOIMMUNE SKIN DISEASES

ABSTRACT OF THESIS
submitted to the Faculty of Medical Sciences, University of Delhi
for fulfilment of requirements for the award of Degree of
DOCTOR OF PHILOSOPHY in MICROBIOLOGY

by

SAJAD AHMAD DAR

Under the guidance of

Supervisor

Prof. Shukla Das
Department of Microbiology,
University College of Medical Sciences (University of Delhi)
and G.T.B. Hospital, Dilshad Garden, Delhi - 110 095

Co-supervisors

Prof. V. G. Ramachandran
Deptt. of Microbiology,
UCMS & G.T.B. Hospital,
Dilshad Garden,
Delhi-110 095

Prof. S. N. Bhattacharya
Deptt. of Dermatology & STD,
UCMS & G.T.B. Hospital,
Dilshad Garden,
Delhi-110 095

Prof. B. D. Banerjee
Deptt. of Biochemistry,
UCMS & G.T.B. Hospital,
Dilshad Garden,
Delhi-110 095

Forwarded by

Head, Department of Microbiology,
Faculty of Medical Sciences, University of Delhi

Abstract

Background: Autoimmune diseases in susceptible individuals depend largely on multiple factors – the genetic makeup, environmental antigen challenge and the immunological status of the afflicted. There is a paucity of knowledge on the risk posed by various environmental toxins, microbial antigen or superantigen stimulants which may modify the clinical expression of the autoimmune skin diseases and the response of the patient to treatment strategies. The participation of cytokines in the induction and effector phases of the immune and inflammatory response shows that their polymorphism may be playing a critical role in the development of autoimmune diseases.

Objectives: Present study is an attempt to elucidate the role of various microbial antigens or superantigens (sAgs) produced by certain pathogens, and organochlorine pesticides (OCPs) in altering effector T cells and cytokine expression in patients of three autoimmune skin diseases viz., pemphigus, systemic sclerosis (SSc) and systemic lupus erythematosus (SLE). Efforts were also made to investigate single nucleotide polymorphism of an array of cytokines at DNA/genomic level to enhance our understanding of their role in disease progression in these autoimmune skin diseases.

Methods: Fifty three patients diagnosed to have autoimmune skin disease – pemphigus (20), systemic sclerosis (20) and systemic lupus erythematosus (13) were enrolled in the study. Peripheral venous blood (~10 ml) was collected aseptically from each patient and used for isolation of peripheral blood mononuclear cells (PBMCs), pesticide residue quantification and DNA extraction. Six months after induction of clinical remission by immunosupressives/steroids, repeat blood samples were collected from each patient for PBMCs isolation. Healthy age-matched volunteers were also enrolled for comparisons. PBMCs were stimulated *in-vitro* with different concentrations of microbial antigens or sAgs which included Staphylococcal enterotoxin B (SEB), Streptococcal pyrogenic exotoxin A (SPEA) and Cytomegalovirus (CMV) antigen; recall antigens (rAgs), *Candida albicans* antigen (CA) and purified protein derivative (PPD) from *Mycobacterium tuberculosis*; and OCPs, hexachlorocyclohexane (HCH) and o,p'-dichlorodiphenyltrichloroethane (DDT). After stimulation, cells were stained with different dye labeled monoclonal antibodies for analysis of various T cell subsets by flowcytometry. Quantitative cytokine analysis in PBMCs culture supernatants was done

by ELISA. Cytokine genotyping was carried out by polymerase chain reaction using sequence specific primers.

Results: Significant elevation in $CD3^+CD4^+$ T cells and expression of the memory ($CD45RO^+$) markers on these cells was observed in PBMCs of all the three diseases with SPEA and SEB stimulation as compared to levels in unstimulated cells. However, SSc and SLE patients PBMCs also showed a significant increased in $CD4^+CD45RA^+$ T cells. Further, an increase in the production of Th1 (IL-2 and IFN-γ) cytokines by PBMCs of all the diseases was observed with sAgs. On the other hand increased levels of IL-4 were also seen in SSc and SLE patient PBMCs. CMV stimulation resulted in an increase in CD4:CD8 ratio and also in elevation of memory population of both $CD4^+$ and $CD8^+$ T cell pool in all the diseases as compared to healthy controls. Moreover, IL-4 in pemphigus, IL-4 and IFN-γ in SSc, and IL-4, IFN-γ and IL-2 in SLE patient PBMCs were found to be significantly raised with CMV stimulation. Recall antigens were able to increase $CD4^+CD45RO^+$ population of T cells in pemphigus PBMCs only. However, decrease in IL-10 was observed in all the three diseases with these antigens. Levels of HCH (β-isoform) and DDT metabolite (p,p'-DDE) were found to be elevated in the blood of all the three disease groups. Exposure of PBMCs to these OCPs mainly caused reduction in $CD8^+$ T cells particularly naïve ($CD45RA^+$) ones, and also in $CD4^+CD25^+$ T cells, a cell population containing T regulatory cells. Upon OCPs treatment, PBMCs showed a decrease in Th1 cytokine levels in pemphigus, but an increase in Th2 cytokine production in SSc and SLE. Effect of immunosuppressive therapy was mostly evident on naïve $CD4^+$ T cells, registering a decrease in CD4:CD8 ratio in all the diseases, after *in-vitro* stimulation with most of the antigens/stimulants. However, in general, an increase in memory $CD4^+$ T cells was conspicuous. Decreased Th1 cytokine response, on the whole, was observed post-therapy in all the diseases; but no significant change in Th2 response was observed except a decrease in IL-10 with sAgs in SSc and SLE. Cytokine genotyping showed significant correlation of IL-1R1, IL-4Rα, IFN-γ and TGF-β1 SNPs with pemphigus; IL-1β and TNF-α SNPs with SSc; and a weak association of TGF-β1 and IL-10 with SLE.

Conclusions: The study indicates that host factors influence the magnitude of cytokine responses to sAgs and consequently the clinical outcome of the infection which in turn suggests an important role for sAg-activated T cells and cytokines in triggering episodes

of clinically active autoimmunity in these patients. An attempt to contain colonizing and or sAg-producing microbial agents may be worthwhile, and may decrease the severity by inhibiting such triggers and prevent frequent relapses. High pesticide levels in the blood can trigger or worsen the immunological defects by causing T cell suppression in these patients. When there is substantial suppression of T and B cell function, there is increased susceptibility to infection. We therefore speculate that the systemic absorption of the pesticide after the topical contact may be one of the factors triggering the immunological mechanisms in these diseases. The ability of the immunosuppressives/steroids to influence specifically, $CD4^+$ T-cell subsets may play a central role in controlling these disorders in humans. Impaired production of cytokines due to genetic variations might favor infections and production of autoantibodies in these autoimmune skin diseases.