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DIFFERENTIAL EXPRESSION OF SURFACE MARKERS CD45RB AND CD44 ON MURINE CD8+ CELLS

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ABSTRACT

Considering the emerging importance of phenotypic markers as indicators of cell function and differentiation, we studied patterns of CD44 and CD45RB expression in CD8+ murine T cells with prior exposure to antigen or staphylococcal enterotoxin B (SEB). Following *in vivo* priming with two purified protein derivatives (one from a virulent WHO strain and the other from an avirulent strain), T cell enrichment was performed on murine spleen cells and followed by *in vitro* restimulation with SEB. Flow-cytometry studies were done to monitor the expression of CD44 or CD45RB. Results indicate that primed or memory CD8+ cells do not display any significant change in the expression of CD45RB. The expression of CD44 exhibited significant variations among unprimed and primed mice and between different antigens. We were also able to see differential expression relevant to the virulence of the bacterial strain. This study suggests that by activation of those populations of CD8+, CD44+ cells, resistance to virulent bacteria occurs.
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INTRODUCTION

CD8+ murine T cells have been shown to play a determining role in the outcome of an immune response and recent works indicate that they also take part in the production of cytokines and thus influence the cytokine profile.[1,2] Resting T cells can be subdivided into naive and primed or memory subsets based on their prior exposure to antigen. In the case of CD4+ and most likely CD8+ cells, antigen stimulation results in the up or down regulation of various cell surface markers which is an indicator of cell activation and can often be used to isolate and characterize the cell populations.

Research indicates that cytokine patterns of CD8+ T cell subsets are similar to their CD4+ counterparts.[3,4] Similar to TH1 and TH2 cytokine patterns, TC1 cells are reported to secrete IL-2, and gamma-INF, while TC2 cells produce IL-4, IL-5, IL-6 and IL-10. Recently, the importance of CD8+ cells in the production and regulation of cytokines has also been emphasized by different researchers.[4]

The CD45 family of cell surface glycoproteins have multiple isoforms (A,B,C) which can be distinguished on the basis of molecular size. Monoclonal antibodies specific for the restricted isotopes reveal the differential expression of CD45 isoforms between functional T cell subpopulations. Exon B dependent epitopes have been specifically useful for defining subpopulations of T cells with functional relevance.[5] Lee and others have reported a heterogeneity in the staining of CD4+ cells with exon B specific monoclonal antibodies dividing them into a $CD45RB^{hi}$ and $CD45RB^{lo}$ sub-populations.[6] $CD45RB^{hi}$ cells were shown to secrete more IL-2 after stimulation and thus were classified as virgin cell populations whereas $CD45RB^{lo}$ were shown to secrete more IL-4 and were characteristic of memory cells.[7]

The high expression of the CD44 (pgp-1) marker on T cells following antigenic stimulation has been reported by Swain, Budd and others to be a potential marker of activation and indirectly related to subset function. It has been noted that the expression of high levels of CD44 on CD4+ cells following stimulation is indicative of memory function while the $CD44^{lo}$ subset is related to a naive precursor population.[8,9]

In this work, we attempt to study murine CD8+ T cells for the expression of two surface markers, CD45RB and CD44 which have been reported to correlate with the state of differentiation of T cells and distinguish between virgin and primed or memory cells. The effect of variant *in vivo* antigenic stimulation and the effect of *in vitro* restimulation

with a superantigen, staphylococcal enterotoxin B (SEB) which has been shown to increase cytokine titers,[13] are also monitored to find out whether differential antigenic stimulation results in changes in the expression of these surface markers. The results indicate a significant difference between the expression of these markers among cell populations primed by different antigens and restimulated by SEB. The present work also reports differential expression of the CD44 high and low subsets subsequent to the administration of two types of purified peptide derivatives from a virulent and a non-virulent strain of *Mycobacterium tuberculosis.*

MATERIAL AND METHODS

Animals

Female, 8-12 weeks old, Balb/c mice were obtained from the Razi Institute, Karaj, Iran. They were fed standard mouse chow and water *ad libitum.*

Immunizations

Mice were primed by intra-peritoneal injections of 100μg of antigen in Incomplete Freund's Adjuvant (IFA). Control groups received IFA alone in saline.

Antigens

Two purified protein derivatives, PPD1 (H37Rv virulent ATCC 27294) and PPD2 (H37Ra avirulent strain) were originally obtained from the WHO strains of *Mycobacterium tuberculosis.* PPD2 was obtained in the form of a purified
ria kindly donated by Collection De Cultures National de Microorganisms, Pasteur Institute, Paris, France.

Staphylococcal toxin B (SEB, Toxin Technology, USA) was used as a superantigen for *in vitro* stimulation.

Cell preparations

Cells were obtained from the spleens of mice sacrificed days after priming with PPD1, PPD2 or IFA. Single cell suspensions were prepared and spleen cells were enriched for T cells using a Collect Immunocolumn System (Alberta, Canada). The method consisted of the adsorption of Ig + B cells to goat anti-mouse IgG coupled affinity column for the purification of T cells. The cells eluted from the columns were > 88% T cells as determined by immunofluorescence analysis.

Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum, penicillin 100 U/mL and streptomycin 100 mg/mL. 96-well flat bottomed microtiter plates (Nunc, Denmark) were used for *in vitro* stimulation of cells. SEB 50 μg/mL was added to cell cultures and 48 hrs later, cells were assayed by flow cytometry.

Flow cytometry

Cells were assessed by two color immunofluorescence with FITC- conjugated monoclonal anti-CD44 (pgp-1) and anti-CD45RB antibodies and the biotinylated anti-CD8a detected with streptavidin phycoerytrin (all from Pharmingen, USA). Class matched irrelevant antibodies were used as controls. Cells were suspended at 10^3 cells/mL and incubated with the appropriate antibody on ice for 30 min, washed twice and analyzed by flow cytometry using an Elite Coulter. The lymphocyte fraction was defined by gating based on forward and side scatter and the percentage of positively stained cells and the bright (high) and dull (low) populations were determined by a cytometer.

Statistical calculations were performed using the unpaired Student's t-test and significance
95% level.

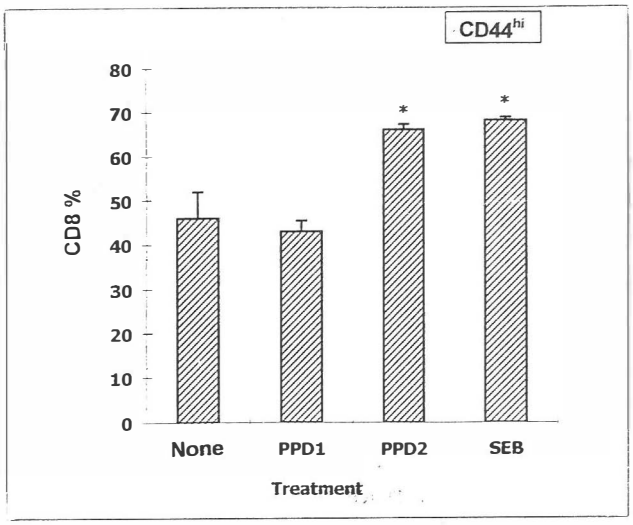

Fig. 1. Expression of $CD44^{hi}$ on CD8+ cells:
a) None: Primed with only IFA (46% + 6)
b) Primed with PPD1 (43% + 2.4)
c) Primed with PPD2 (66.9% + 1.2)
d) Primed with IFA and restimulated with SEB (63.1% + 0.7)
* indicates statistical significance.

RESULTS

PPD1 and PPD2 primed CD8+ cells were studied for their expression of CD45RB and CD44 before and after SEB restimulation *in vitro.* CD8+ populations from mice primed with PPD2 had higher numbers of $CD44^{hi}$ cells, when compared with unprimed CD8+ cells or PPD1 primed cells. When these cells were restimulated with SEB, expressions of CD44 did not increase over the amount expressed with PPD2 which was considerably higher than that of PPD1 or unprimed cells (Figure 1).

Higher percentages of CD8+ cells from mice primed with

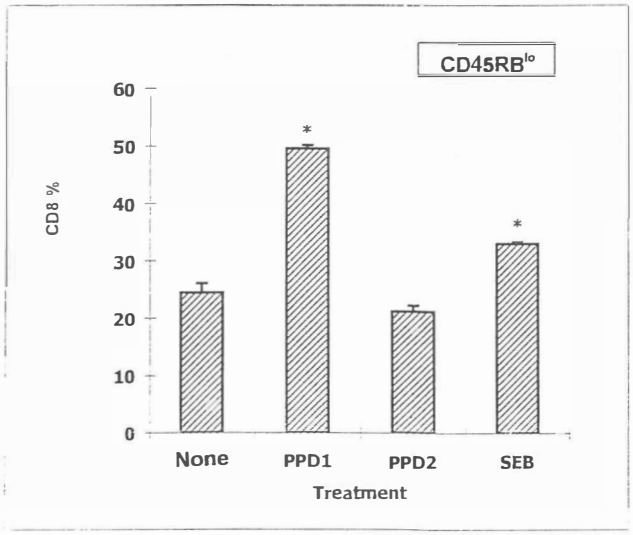

Fig. 2. Expression of $CD45RB^{lo}$ on $CD8^+$ cells:
a) None: Primed with only IFA (24.4% + 1.6)
b) Primed with PPD1 (49.4% + 0.6)
c) Primed with PPD2 (21.2% + 1.0)
d) Primed with IFA and restimulated with SEB (33% + 0.3)
*indicates statistical significance.

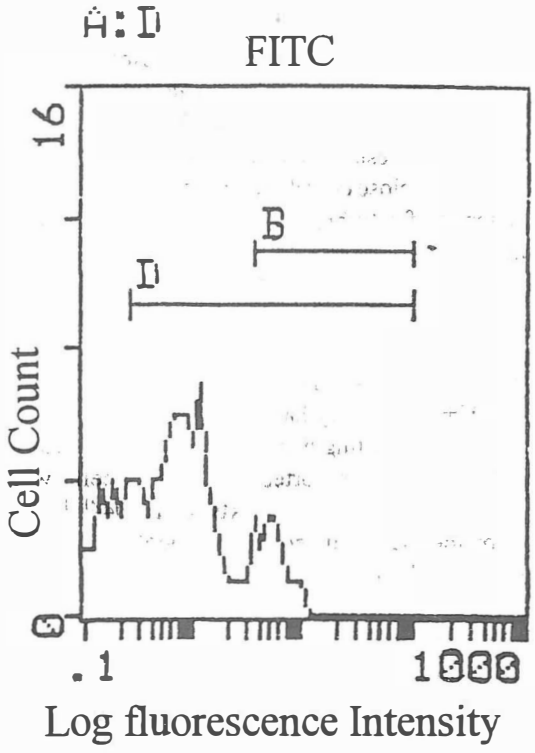

Fig. 3. CD45RB staining pattern of splenic CD8+ cells. CD8+ enriched splenocytes from Balb/C mice were stained with FITC-conjugated anti-CD45RB antibodies and biotinylated anti-CD8a (detected with streptavidin phycoerytrin). The cells were sorted into $CD45RB^{lo}$ (D-B) and $CD45RB^{hi}$ (B).

PPD1 were $CD45RB^{lo}$ when compared to cells from unprimed mice or cells from PPD2 primed mice.

All antigen stimulated CD8+ cells expressed a majority of $CD45RB^{hi}$ cells (69.3% + 1.3) but SEB stimulated populations had higher $CD45RB^{lo}$ percentages (33% + 0.3) as compared to unstimulated cells (24.4% + 1.6).

Our results showed higher expressions of $CD45RB^{lo}$ in PPD1 primed cells as compared to PPD2 or control (Figure 2). However, SEB restimulation did not increase $CD45RB^{lo}$ on the cells more than priming with PPD1 but it was higher than control or PPD2 primed cells (Figure 2).

DISCUSSION

The results presented here indicate the differential expression of surface markers of cells from primed or unprimed mice stimulated or unstimulated *in vitro*. Studies on the cytokine profiles and expression of surface markers for CD8+ cells has shed light on the role of these cells in health and disease. While certain studies on the cytokine patterns of CD8+ subsets report a similar pattern to their CD4+ counterparts, others indicate that CD8+ cells may exert a regulatory role by differential cytokine production. Correlations between cytokine patterns and naive or effector status of cells and the expression of CD44 and CD45 and other markers such as CD30 have been cited in the literature.[6-10]

Studies on the selection mechanism of T1/T2 cytokine patterns have continued vigorously during the past years. The discovery that naive CD4+T cells or THO cells produce TH1 and TH2 cytokines suggests that selective differentiation induced in these cells by ligand could determine the functional outcome of a given immune response.[11,12]

Research has shown that altering the sequence of the peptide component of the TCR T-cell receptor ligand can affect the ensuing cytokine profile and immune response against the peptide.[18] Our studies also indicate that varying antigen epitopes can induce different cytokine responses in similar circumstances.[13]

Antigen dose, MHC-TCR interaction, MHC genotype ligand density on B cells and costimulatory signals have all been shown through experiments to influence or elicit TH1 or TH2 immunity.[14,15]

Our studies on CD8+ populations indicate that the two peptides PPD1 and PPD2, one of which is not a pathogen (PPD2) and one which is clearly a virulent agent (PPD1), can induce different levels of expression of differentiation markers. In fact, it is possible that the virulence of PPD1 is a reflection of lower expressions of CD44 on CD8+ cells.

Our study also indicates that the superantigen SEB which is known to activate the TCR through unconventional mechanisms can elicit a differential expression of these

markers.

In our initial study we endeavored to clarify the role of antigen epitopes in shifting the cytokine response.[13] Our studies indicated that the antigen has a role in determining the outcome of the cytokine response. A comparison between those ELISA results and the expression of surface markers indicates a close correlation between these two parameters in terms of a differential response to varying antigenic epitopes.

Other researchers have worked on the expression of surface markers in various states of health and disease. Certain workers have concluded that antigen inexperienced naive CD8+ cells express $CD45RB^{hi}$ and $CD44^{lo}$ with a pattern similar to CD4+ cells. They have concluded that memory CD8+ T cells can be distinguished only by high expression of CD44. They have also reported that CD8+ T cells, when restimulated *in vitro,* express $CD45RB^{hi}$ and $CD44^{hi}$.[1,16]

Studies on the THl mediated colitis model show that the disease is induced by transfer of $CD45RB^{hi}$ and prevented by the co-transfer of $CD45RB^{lo}$.[11,17-18]

In our study the avirulent PPD2 primed CD8+ cells expressed more $CD44^{hi}$ indicative of an effector or memory state as other workers have reported. The PPD1 primed CD8+ cells and the unprimed cells expressed lower levels of $CD44^{hi}$. SEB stimulation also resulted in more $CD44^{hi}$ cells. Since T-cells were shown to produce more IL-5 after PPD1 priming and restimulation, this can be attributed to a shift to the type 2 pattern and therefore more expression of $CD44^{hi}$ may be indicative of the T2 pattern.

The majority of stimulated CD8+ cells expressed $CD44^{hi}$ but those re-stimulated with SEB had higher $CD45RB^{lo}$ (33%) compared to control or PPD2. This can be indicative of a T2 cytokine pattern as mentioned above.

This study indicates that changes in CD8+ cell marker expression may correlate with cytokine patterns and immune status. The correlation between cytokine patterns and disease progression is now well known for many pathological conditions. Insight into the ontological and functional status of CD8+ T cells may find more relevance as the molecular basis of disease states is revealed.

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