

T-CELL RECEPTOR V β 8.1 PEPTIDE REDUCES COXSACKIEVIRUS INDUCED
CARDIOPATHOLOGY DURING MURINE ACQUIRED IMMUNODEFICIENCY
SYNDROME AND AGING

By

Ramón Tomás Sepúlveda

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Ramon Tomas Sepulveda

entitled T-Cell Receptor V β 8.1 Peptide Reduces Coxsackievirus Induced Cardiopathology During Murine Acquired Immunodeficiency Syndrome and Aging

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

John J. Marchalonis Ph.D. Date: 11-23-05

Claire Payne Ph.D. Date: 11-23-05

Nafees Ahmad Ph.D. Date: 11-23-05

Harris Bernstein Ph.D. Date: 11-23-05

Ronald R. Watson Ph.D. Date: 11-23-05

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copies of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Date: 11-23-05

Dissertation Director: John J. Marchalonis Ph.D.

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SIGNED: Ramon Tomas Sepulveda

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In walking this path to accomplish this goal
Not a step was taken without you at my side
Not a tear was dropped without you to help it subside
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It's only You that lives deep inside.

Te Amo.

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ABSTRACT

Infection of people with human immunodeficiency virus (HIV) as well as LP-BM5 infection in mice results in progressive deterioration of the immune system in the majority of untreated hosts. Peptide immunotherapy has been shown to be effective in the stimulation or immunoregulation of T-helper 1 (TH1) and T-helper 2 (TH2) response subsets. In murine acquired immunodeficiency syndrome (AIDS), TH1 deficiency enables the host to be susceptible to coxsackievirus infection, inducing cardiopathology in a short period. T-cell receptor (TCR) V β 8.1 peptide, a 16-mer peptide containing the entire CFR1 segment and part of the FR2 region of human V β 8, showed both an immunoregulating and immunostimulating effect in murine AIDS. TCR V β 8.1 peptide acts on T cells promoting interleukin-2 production and therefore enhancing a cell-mediated immune response. It retarded development of cardiopathology due to coxsackievirus infection. Retrovirus infected mice treated with the peptide showed a longer life span than the nontreated retrovirus infected animals.

CHAPTER 1

INTRODUCTION

1.1 Background

Humans and mice naturally have constitutive levels of autoantibodies against their own T-cell receptor V β domains (Marchalonis et al., 1994). The levels of these autoantibodies are markedly increased by retroviral infection, such as HIV-1 in humans (Lake et al., 1994; Marchalonis et al., 1997), and in infection of C57Bl/6 mice with defective murine leukemia virus. A major epitope recognized is defined by a synthetic 16-mer peptide encompassing the entire V β CDR1 segment and a portion of the second framework (Marchalonis et al., 1992a). Autoantibodies to this peptide-defined epitope are also elevated in autoimmune diseases, particularly IgM autoantibodies in rheumatoid arthritis and IgG molecules in systemic lupus erythematosus (Marchalonis et al., 1994; Marchalonis et al., 1993a; Marchalonis and Schluter, 1993). The peptide epitope was a somewhat unusual one in the sense that all vertebrates tested, including rabbits (Marchalonis et al., 1993) and sharks (Marchalonis et al., 2001) possess natural antibodies to this marker, but attempts to induce antibodies, even by coupling the peptide carriers and immunizing in adjuvant failed to generate increased titers relative to the background levels. Since the TCR β genes of humans and mice are orthologous (Koop and Hood, 1994), it was possible to use the same peptides in the two distinct vertebrate species. Several of our studies were designed to determine whether administration of the peptide prior to infection of mice with the LP-BM5 leukemia retrovirus mixture

(prophylactic), or following administration (therapeutic), would have an effect on the development of immunosuppression generated as a consequence of the retroviral infection (Watson et al., 1995). Also, studies have been done to determine the in vitro reactivity of the peptides with T-cell receptors and their capacity to activate T and B lymphocytes in culture as well as in vivo studies on the effects of immunomodulatory TCR V β -derived peptides on cytokine production, function, and resistance to opportunistic infections.

1.2 HIV/AIDS

Acquired immune deficiency syndrome (AIDS) is a clinical disorder representing the end point in a progressive sequence of immunosuppressive changes that render the body highly susceptible to tumors and opportunistic infections. Because of the unique pathogenicity of human immunodeficiency virus (HIV) and its long latent period the AIDS epidemic is expected to steadily increase in the United States, with the number of deaths increasing at a proportional rate. Furthermore, immune and other physiological defects induced by HIV infection appear to be progressive and irreversible, with a high mortality rate.

AIDS was first identified in the USA in 1981. The epidemic has now spread to every part of the USA and to all sectors of society.

It is thought that more than one million people are living with HIV in the USA and that more than half a million have died after developing AIDS.

Unfortunately, American HIV surveillance is far from comprehensive so many statistics must be based on reports of AIDS diagnoses. In interpreting such AIDS statistics, it is important to remember that they do not correspond to new HIV infections.

According to estimates from the UNAIDS/WHO AIDS Epidemic Update (December 2004), 37.2 million adults and 2.2 million children were living with HIV at the end of 2004. This is more than 50% higher than the figures projected by WHO in 1991 on the basis of the data then available.

During 2004, some 4.9 million people became infected with the human immunodeficiency virus (HIV), which causes AIDS.

The year also saw 3.1 million deaths from AIDS - a high global total, despite antiretroviral (ARV) therapy which reduced AIDS-related deaths in the richer countries. Deaths among those already infected will continue to increase for some years even if prevention programs manage to cut the number of new infections to zero. However, with the HIV positive population still expanding the annual number of AIDS deaths can be expected to increase for many years unless more effective provision of ARV medication begins to slow the death rate.

The total number of people living with HIV continues to rise in high-income countries, largely due to widespread access to antiretroviral treatment, which prolongs the lives of HIV+ people. This causes an increase in the pool of HIV+ people who are able to transmit the virus onwards. It is estimated that 1.6 million people are living with HIV in North America and Western Europe - a figure that includes 65,000 who were newly infected in 2004.

AIDS claimed approximately 22,500 lives in 2004, although the rate of AIDS related deaths is continuing to decline as ARV medicines are made increasingly available. There is mounting evidence that prevention activities in several high income countries are not keeping pace with the spread of HIV and that in some places they are falling behind. Such shortcomings are most evident where HIV is found mainly among marginalized groups of the population such as drug users, immigrants and refugees.

1.3 LP-BM5 Retrovirus

Animal model systems that simulate many aspects of the human AIDS yet have the benefits of large numbers of subjects, low cost, and a well defined immunological system are critically needed for mechanistic studies and development of therapeutics. There are no doubts that knowing in one model system the precise cellular and molecular mechanisms that lead to immunodeficiency would represent a significant step forward in the understanding of retrovirus induced immunodeficiency in general. Retroviruses appear to be widespread and have been isolated from fish, mammals, birds, and reptiles.

However, one of the most used retroviral agents to study AIDS and its related therapeutics is murine AIDS, caused by LP-BM5 murine leukemia retrovirus (MuLV) infection in a genetically susceptible strain of female C57BL/6 mice.

Recent advances in both murine AIDS and early stage of human AIDS are strikingly similar in many respects, including changes in immune function, cytokine production, T-cell differentiation, disease resistance, and oxidative stress. The pathogenic mechanisms underlying HIV infection and AIDS are not unidimensional but rather are extremely complex. As the key to understanding HIV/AIDS pathogenesis lies in elucidating the course of infection and the virus-host relationship in the years preceding terminal illness, use of animal models is essential for a comprehensive understanding of the pathogenesis of HIV/AIDS.

The virus mixture LP-BM5 MuLV (lymphoproliferative murine leukemia virus), which was originally isolated by Latarjet and Duplan, produces non-neoplastic lymphoproliferative disease in adult mice (Latarjet et al., 1962). LP-BM5 MuLV was obtained from a nonthymic lymphoma that had been induced by irradiation of mice. Then bone marrow stromal cells were isolated from the infected mice and grown as cell lines that yielded the agent. The cluster of MuLV viruses includes (a) a 4.8-kbp replication-defective BM5d, (b) a B-tropic mink cell focus-inducing virus (MCFV), and (c) a replication-competent B-tropic ectropic virus (BEV). The immunological disorders are apparently induced by defective genome BM5d, but not after infection by BEV and MCFV, which serve to facilitate the transmission of BM5d defective virus in mouse tissues. The murine major histocompatibility complex (MHC) has been shown to

influence the development of murine AIDS and the extent of virus spread in the adult mouse (Makino et al., 1987). This murine retrovirus infection provokes an enlargement of lymphoid organs, primarily spleen and lymph nodes. Although lentiviruses are associated with murine AIDS and C-type retroviruses appear to be the causative agents in human AIDS, both are very similar in that they cause pronounced immunosuppression as well as reduced resistance to opportunistic pathogens and neoplasia in their respective hosts. It is well established that the development of disease reflects complicated interactions among cells of the immune system. The major immune cell targets for LP-BM5 MuLV and HIV are B cells and macrophages. CD4⁺T cells and B cells are required for the development of murine AIDS infection and the associated immunosuppression (Cerry et al., 1990; Yetter et al., 1988).

In the murine AIDS model the pathogenesis of LP-BM5 MuLV is also characterized by immune dysfunction with many changes similar to those reported in human HIV infection (Gardner, 1990). Although T cells are not the main source of murine viral replication they are essential for early pathogenesis and disease (Mosier, 1986). The temporal sequence of T-cell function changes are similar between murine and human AIDS, with Th-cell function lost well before dysfunction of cytotoxic T-cells (Watson, 1989). The quantitative loss of overall T-cell function occurs far in advance of the reduction in CD4⁺ Th-cell numbers (Watson, 1989). However, the extent of Th-cell depletion varies from organ to organ. CD4⁺ Th-cell populations shift from a heterogeneous phenotype with characteristics of a mixed population of naïve and memory cells, to a more homogeneous phenotype that broadly shares characteristics of

both memory and activated cells (Muralidhar et al., 1992). The initial resting immunocompetent CD4⁺ Th-cell population, in other words, is replaced by a polyclonal population described as being in an anergic state (Muralidhar et al., 1992). The state of anergy has been demonstrated to be related to defects in the signal transduction pathway (protein kinase C, calcium mobilization, and phospholipase C) induced by LP-BM5 MuLV during murine AIDS (Chattopadhyay et al., 1989; Fitzpatrick et al., 1992; Mosier, 1986; Muralidhar et al., 1992). Furthermore, the state of anergy is apparently induced by a soluble, diffusible, LP-BM5 MuLV-encoded SAG component that is capable of stimulating most CD4⁺ Th cells (Fitzpatrick et al., 1992; Muralidhar et al., 1992).

In murine AIDS, LP-BM5 retrovirus also induces an early phase of B-cell hyperactivity and polyclonal activation (Morse et al., 1988). B cells and their production of Ig show a significant number of alterations due to retroviral infection (Morse et al., 1988). Activation of both T and B lymphocytes can be detected within 1 week of infection, as determined by flow cytometric analysis of the size of T and B cells and measurements of the percentage of B cells secreting IgM. There is an absolute increase in the number of B cells in the spleen and lymph nodes, and a threefold increase in the fraction of B cells in cell cycle (Mosier et al., 1985). Although T cells from LP-BM5 MuLV-infected mice will not provide helper activity to normal B cells for a specific antibody response *in vitro*, they do stimulate elevated polyclonal IgM secretion by normal B cells (Mosier et al., 1985). During the early stages of the LP-BM5 MuLV infection, B cells produce large amounts of Igs with increased number of plasma or Ig-producing cells. There is widespread, polyclonal IgM secretion by normal B cells (Mosier

et al., 1985). The later stages of murine AIDS involve continued expansion of members of the B lymphocyte lineage, including all stages from pre-B cells to plasma cells, increased numbers of the monocyte-macrophage lineage, and appearance of immature T cells in the periphery (Holmes et al., 1990). However, there is a substantial loss (>90%) of T and B cells in the intestinal mucosa. Such local losses may explain the increased colonization and loss of intestinal resistance to opportunistic pathogens common to human AIDS patients.

NK-cell cytotoxicity is also reduced in murine AIDS, but lymphokine activated killer (LAK) cell function is maintained for 8-10 weeks after initiation of LP-BM5 MuLV infection (Soave, 1988). Peritoneal macrophage number is reduced, and their monokine release is impaired by retrovirus infection during murine AIDS. Diminished *in vitro* pathogen killing of macrophages and decreased numbers of activated macrophages have also been observed during murine AIDS (Morse et al., 1988). In addition, spontaneously increased prostaglandin E and IL-1 secretion during murine AIDS was reported (Morse et al., 1988).

1.4 Coxsackievirus

Picornaviruses are small, nonenveloped viruses containing a single positive sense RNA strand. The capsid is comprised of 60 protomers, each containing a single copy of the four structural proteins VP1 to VP4. Proteins VP1, VP2 and VP3 are primarily involved in producing the outward topography of the capsid. Protein VP4 is an internal capsid protein and is located beneath the other three virus proteins. The role of VP4 is not

absolutely clear, but it probably plays a dual role in stabilizing the interactions/tertiary structure of the other capsid proteins and facilitating the interactions between the capsid and viral genome. The basic picornavirus replication cycle can be divided into (1) virus attachment to the cell membrane, (2) virus penetration into the cytoplasm, (3) uncoating of the genome, (4) replication, (5) assembly of progeny virions, and (6) release of progeny virions into the extracellular space. Virus attachment occurs through binding specific membrane components (CAR: coxsackievirus-adenovirus receptor) primarily to the groove regions of the viral capsid.

The World Health Organization statistics (1975-1985) rank coxsackie B virus infection as the number one viral pathogen responsible for cardiovascular disease. Coxsackievirus B3 (CVB3) can induce an inflammatory disease in the heart that results in cardiopathology in individuals with compromised immune systems. Certain inbred strains of mice such as the C3H and BALB/C are susceptible to the infection (Gauntt et al., 1979; Huber and Lodge, 1986) while the C57Bl/6 strain is resistant (O'Donoghue et al., 1990; Sepulveda et al., 2002; Sepulveda et al., 2002; Sepulveda et al., 2003). When the immune system is compromised heart pathology will develop in mice that are normally resistant to infection. If the C57Bl/6 strain of mouse is exposed to the CVB3 virus under conditions which compromise immunity such as oxidative stress or retroviral infection, the mice will develop heart disease. Furthermore, sustained cocaine or alcohol use combined with retroviral infection exacerbate the coxsackieviral co-infection leading to more extensive cardiac damage (Sepulveda et al., 2002; Sepulveda et al., 2003).

1.5 TCR V β 8.1 peptide

Human and mice naturally have constitutive levels of autoantibodies against their own T-cell receptor V β domains (Marchalonis et al., 1994). The levels of these autoantibodies are markedly increased by retroviral infection, such as HIV-1 in humans, (Lake et al., 1994; Marchalonis et al., 1997) and in infection of C57BL/6 mice with defective murine leukemia virus, the murine AIDS model (Marchalonis et al., 1994; Dehghanpisheh et al., 1995). A major epitope recognized is defined by a synthetic 16-mer peptide encompassing the entire V β CDR1 segment and a portion of the second framework (Marchalonis et al., 1992). Autoantibodies to this peptide defined epitope are also elevated in autoimmune diseases like rheumatoid arthritis and systemic lupus erythematosus (Marchalonis et al., 1994, 1993a, 1993b). Since the TCR β genes of humans and mice are orthologous, (Koop and Hood, 1994) it was possible to use the same peptides in the two distinct vertebrate species.

Humans infected with HIV-1 have elevated levels of autoantibodies (both IgM and IgG) (Lake et al., 1994; Marchalonis et al., 1997; Marchalonis et al., 1999) to the variable domains of human TCRs.

Peptide TCR V β 8.1 corresponds to the complete CDR1 segment and five residues of the second framework. In a study where 10 HIV-infected individuals were followed for a 2-year period, they showed elevated levels of autoantibodies to the V β 8.1 peptide, which were maintained throughout the period under consideration (Marchalonis et al., 1997).

Experimental infection of C57BL/6 female mice with a defective retrovirus mixture (LP-BM5) induces a condition termed murine AIDS in which there is initially a hyperactivity of the immune system as manifested by lymphadenopathy, splenomegaly, and increased production of immunoglobulin (Liang et al., 1996; Morse et al., 1992). This hyperactivity diminishes and the mice subsequently become immunodeficient in the sense that they are unable to resist infection by opportunistic parasites, they have decreased T- and B-cell function as judged by the capacity for mitogenesis, and their CD4+ T cells undergo a functional shift from Th1- to Th2-type activities (Watson et al., 1995; Gazzinelli et al., 1992; Huang et al., 1993). The injection of this peptide after development of significant cytokine dysregulation, moreover, did not slow the development of murine AIDS (Watson et al., 1995; Liang et al., 1996a, 1996b; Liang et al., 1997a, 1997b; Liang et al., 1998; Liang et al., 2000). Thus, the TCR peptide prevented retroviral immune dysregulation rather than overcoming it once it became predominant. Oxidative damage is increased in human and murine AIDS, perhaps because of the increased inflammatory cytokines. Overall, the effect of the TCR V β 8.1 peptide is largely to reverse the immunosuppressive effects caused by the retrovirus by way of normalizing cytokine dysregulation induced by infection with the LP-BM5 retrovirus mixture.

CHAPTER 2

T-CELL RECEPTOR V β 8.1 PEPTIDE REDUCES COXSACKIEVIRUS INDUCED CARDIOPATHOLOGY DURING MURINE AIDS

Treatment with TCR V β peptide modulated the dysregulation of the immune system seen as a result of LP-BM5 retroviral infection. This study investigated whether TCR V β 8.1 peptide treatment can effectively modulate the progression to murine AIDS and the development of heart pathology when the mice are coinfecting with a secondary infection, CVB3.

2.1 Materials and Methods

2.1.1 Animals and murine AIDS

Female C57BL/6 mice, 4 weeks old, were obtained from the Charles River Laboratories Inc. (Wilmington, DE). Animals were cared for as required by the University of Arizona Committee on Animal Research. After 2 weeks housing in the Central Animal Facility, University of Arizona, they were randomly assigned to one of the following treatments group: (1) uninfected normal mice injected with MCG3 (a synthetic peptide containing the CDR1 sequence of MCG- λ L chain) control peptide, (2) uninfected normal mice injected with MCG3 and infected with CVB3, (3) uninfected normal mice injected with TCR V β 8.1 peptide, (4) uninfected normal mice injected with TCRV β 8.1 and infected with CVB3, (5) LP-BM5-infected mice injected with control peptide (MCG3), (6) LP-BM5-infected mice injected with control peptide (MCG3) and super infected with CVB3, (7) LP-BM5-infected mice injected with TCR V β 8.1 peptide

and super infected with CVB3. Administration of peptides (200 µg/mice in saline, i.p.) was performed twice, on days 14th and 45th after retrovirus infection. A different set of mice with the same group arrangement was considered for the survival study. After 90 days of retroviral infection, the appropriate groups for both sets of mice were co-infected with CVB3. One set of mice were sacrificed for immunological analysis and the second set was used for the survival study.

LP-BM5 retrovirus was administrated intraperitoneally to mice in 0.1 ml saline with an esotropic titre (XC) of 4.5 log₁₀ plaque-forming units (PFU) ml, which induces disease with a time course comparable to that previously published (Sepulveda et al., 2000). Infection of these mice with LP-BM5 retrovirus leads to the rapid induction of clinical symptoms with virtually no latent phase.

2.1.2 Peptides

A set of overlapping 16-mer peptides that duplicate covalent structure of the VBDBJBCβ protein predicted from a human TCR-Vβ sequence has been produced (Marchalonis et al., 2001b). The complete range of peptide sequences was previously reported in detail (Marchalonis, 2001b). Here, we focus on the sequence C K P I S G H N S L F W Y R Q T that corresponds to the complete CDR1 and N-terminal five residues of Fr2 of the human Vβ8.1 gene product (Marchalonis, 2001b). As a control peptide, we used a 16-mer corresponding to the CDR1 of the L chain MCG, because the LP-BM5-infected mice did not produce autoantibodies (Aab) to this peptide. Its sequence is T G T S S D V G G Y N Y V S W Y. The peptide preparations were free of endotoxins. We

have shown previously that normal polyclonal IgG pools contain natural auto-antibodies (Aab) against peptide segments corresponding to CDR1, Fr3, and a constant region loop peptide of the TCR V β -chain.

2.1.3 ELISA for Cytokines

After 100 days of retroviral infection, the mice were humanely euthanized by cervical dislocation following CO₂ inhalation. The production of interleukin (IL)-2, and interleukin (IL)-6 from mitogen-stimulated splenocytes was determined as described previously (Sepulveda et al., 2001). Briefly, spleens were gently teased with forceps in culture medium (CM; RPMI-1640 containing 10% fetal bovine serum, 2 mmol/L glutamine, 1 X 10⁵ U/L of penicillin and streptomycin), producing a suspension of spleen cells. Red blood cells were lysed by addition of a lysis buffer (0.16 mol/L ammonia chloride Tris buffer, pH 7.2) at 37°C for 3 minutes. Next, these cells were washed twice with culture media (CM). Cell concentrations were counted and adjusted to 1 X 10¹⁰ U/L. Splenocyte viability was >95% as determined by trypan blue exclusion. Splenocytes [0.1 ml/well (1X 10¹⁰ cells/L)] were cultured in triplicate on 96-well flat-bottom culture plates (Falcon 3072, Lincoln Park, NJ, U.S.A.) with CM. The splenocytes were then stimulated with concanavalin A (Con A, 1 X 10⁻² g/L, 0.1 ml/well, Sigma, St. Louis, MO, U.S.A.) to determine their production of IL-2 after 24 hours incubation in a 37°C, 5% CO₂ incubator. Splenocytes were also incubated for 24 hours after the addition of lipopolysaccharide (LPS, 1 X 10⁻² g/L, Gibco, Grand Island, NY, U.S.A.) to induce IL-6 production. After incubation, the plates were centrifuged for 10 minutes at 800 x g.

Supernatants were collected and stored at -70°C until analysis. The cytokines were determined by sandwich ELISA using a commercial Endogen kit (Woburn, MA, U.S.A.), as described in previous studies (Sepulveda et al., 2001).

2.1.4 Mitogenesis of Splenocytes

Splenic T- and B- cell proliferation was determined by ^3H -thymidine incorporation as described previously (Sepulveda et al., 2002). Briefly, splenocytes from retrovirus-infected mice were suspended (1×10^6 cells/ml) in 0.1 ml of RPMI 1640 media supplemented with 300 mg/L of L-glutamine, 100Units/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin. Every sample was cultured in triplicate in 96-well flat-bottomed culture plates (Falcon, NJ) with Con A or LPS and/or peptide. Concentration of Con A and LPS used was 2 $\mu\text{g}/\text{mL}$. Cells were cultured for 48 hours (Con A, for T-cell induced cell proliferation or LPS, for B-cell induced cell proliferation) at 37°C in a humidified 5% CO_2 incubator. After 48 hours, cultures were pulsed with ^3H -thymidine (0.5 μCi / well, PerkinElmer life sciences, Boston, MA) and 4 hours later, cultures were harvested using a cell harvester (Cambridge Technology, Cambridge, MA). Radioactivity was determined by a liquid scintillation counter (Tri-Carb, 2200 CA, Packard, Laguna Hills, CA). Data were presented as counts per minute (cpm).

2.1.5 Survival Study

For the survival study the groups employed were: (1) retrovirus infected; (2) retrovirus infected + TCR V β 8.1 peptide treated; (3) retrovirus + coxsackievirus infected;

and (4) retrovirus + coxsackievirus infected + TCR V β 8.1 peptide treated. Coxsackievirus co-infection occurred at 90 days after retroviral infection when murine AIDS had developed (Sepulveda et al., 2002). Once infected with CVB3, the survival study was initiated, and mice were counted every 5 days ending at day 35 after initial CVB3 infection.

2.1.6 Coxsackievirus B3 infection

Cardiovirulent coxsackievirus B3 serotype 59 stocks were propagated in HeLa cell monolayers in minimal essential medium supplemented with 10% fetal bovine serum and 50 mg/L gentamicin (GIBCO BRL, Gaithersburg, MD) at 37°C in a humidified 5% CO₂ atmosphere. Virus was titered by tissue culture infectious dose-50 (TCID₅₀) (Sepulveda et al., 2005). After 3 months of retroviral infection, mice were inoculated intraperitoneally with 3 x 10⁵ TCID₅₀ of CVB3 strain 59 in 0.1 ml of MEM. Mice were sacrificed after 12 days of CVB3 infection.

2.1.7 Histopathology

At indicated times after inoculation, mice were killed and their hearts removed for study. Hearts were rinsed in saline and transversely cut in half. One half of each heart was immediately placed into Histochoice Tissue Fixative (AMRESCO, Solon, OH, U.S.A.) and stored at room temperature. Fixated hearts were sectioned (6 μ m) on a Zeiss HM 505 N cryostat (Carl Zeiss, Thornwood, NY, U.S.A.) and stained with hematoxylin and eosin.

The severity of inflammatory lesions within the myocardium was graded by a pathologist without knowledge of the other experimental variables. Grading was performed in a semiquantitative manner according to the relative degree (from heart to heart) of mononuclear cell infiltration and the extent of necrosis.

2.1.8 *In vitro* studies

Incubation of splenocytes from retrovirus-infected mice at 10^5 cells/well were stimulated with LPS or ConA as described above. Every sample was determined in triplicate. Doses of TCRV β 8.1 peptide are as indicated in a total volume of 0.3 ml per well. Control peptide was used at concentration of 1 μ g/well. Cells were cultured in RPMI 1640 media supplemented with 10% FBS, 300 mg/L of L-glutamine, 100 Units/ml penicillin, and 100 μ g/ml streptomycin. Cells were harvested at 48 h of incubation at 37°C and 5% CO₂.

Production of IL-2 and IL-6 *in vitro* by splenocytes of healthy and retrovirus infected mice were cultured at 1×10^5 per cells per well in RPMI 1640 supplemented with 10% FBS, 300 mg/ml of L-glutamine, 100 Units/ml penicillin, and 100 μ g/mL streptomycin. Doses of TCR V β 8.1 peptide are as indicated in a total volume of 0.3 ml per well. Every sample was determined in triplicate. β 1 control peptide was used at concentration of 1 μ g/well. Supernatant was retrieved after 5 days of incubation at 37°C and 5% CO₂. Cytokine determination was done as described above.

2.1.9 Statistics

All variables were compared using one-way analysis of variance (ANOVA) for comparison between any two groups. Differences between two groups were considered significant at $p < 0.05$.

2.2. Results

2.2.1 Body and Spleen Weight

There was no change in food consumption or body weight of the mice due to various TCR peptides treatment (data not shown). The spleen weights after LP-BM5 retrovirus infection significantly ($p < 0.05$) increased (data not shown).

2.2.2 Cytokine Production of Splenocytes Treated *in vitro* with TCR peptide

To determine the effect of TCR V β 8.1 peptide on Th1 cytokine production, *in vitro* assessment of IL-2 by splenocytes from retrovirus-infected mice treated with different concentrations of TCR V β 8.1 peptide was determined (Figure 2.1). As the peptide concentration increased so did the production of IL-2, indicating that the peptide modulated T-cell activation. The combination of TCR V β 8.1 peptide with Con A, a T-cell mitogen, did not significantly increase IL-2 production when compared to the effect of the TCR V β 8.1 peptide by itself ($p > 0.1$). The effect of the TCR V β 8.1 peptide on IL-2 production by splenocytes from uninfected control mice has been reported elsewhere (Sepulveda et al., 2002). Both the control peptide and Con A induced minimal production of IL-2.

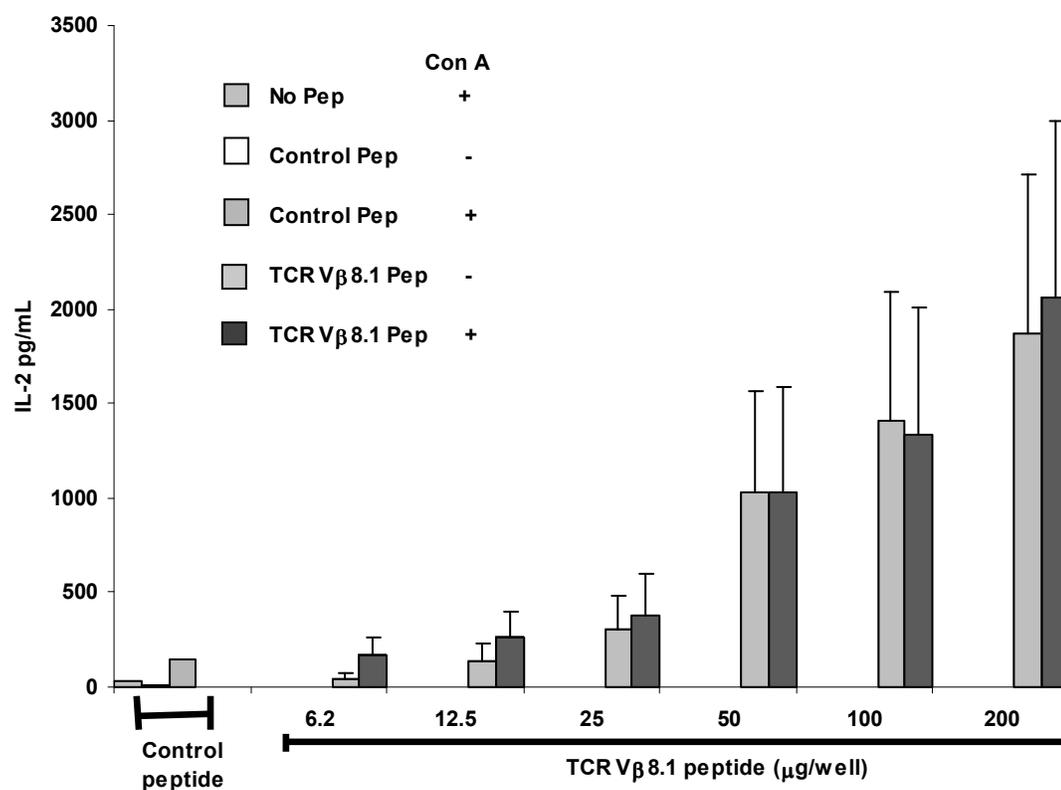


Figure 2.1: In vitro production of IL-2 by Con A and TCR Vβ8.1 peptide stimulated retrovirus infected murine splenocytes. Data for every sample from Con A + TCR Vβ8.1 peptide treated cells were determined in triplicate. Control peptide, Con A and Con A + Control peptide treated cells were determined in quintuplets. Doses of TCR Vβ8.1 peptide are as indicated in a total volume of 0.3 mL per well. Splenocytes were isolated from LP-BM5-infected C57Bl/6 mice and cultured 1×10^5 cells per well in RPMI 1640 media supplemented with 300 mg/L of L-glutamine, 100 Units/mL penicillin, and 100 μg/mL streptomycin. Concentration of Con A used was 2 μg/mL, which did not produce maximum cytokine secretion. Control peptide was used at a concentration of 1 μg/well. Supernatant was retrieved after 5 days of incubation at 37°C and 5% CO₂.

To evaluate the effect of TCR Vβ8.1 peptide on the T_H1/T_H2 cytokine production ratio, IL-2 (Th1) and IL-6 (Th2) cytokines were determined from splenocytes of healthy and retrovirus infected mice. *In vitro* culture of the splenocytes were done with increasing amounts (from 6.25 to 200 μg/well) of peptide (Figure 2.2). For the healthy splenocytes,

Th1/Th2 ratio was maintained between 3 to 4.4, with a slight decrease at a higher concentration of the peptide.

For splenocytes from retrovirus-infected mice, 6.25 $\mu\text{g}/\text{well}$ of peptide reflected a minimum ratio and was increased as the peptide concentration also increased, being at its maximum point at 50 $\mu\text{g}/\text{well}$ of the TCR V β 8.1 peptide. At 100 and 200 $\mu\text{g}/\text{well}$ of the peptide, the ratio began to decrease but was maintained stable at 1.25. The maximum Th1/Th2 cytokine ratio for healthy splenocytes was 4.4 when 12.5 $\mu\text{g}/\text{well}$ of the TCR V β 8.1 peptide was used, while for the splenocytes from retrovirus-infected mice the maximum ratio was 2.2 with 50 $\mu\text{g}/\text{well}$ of the peptide.

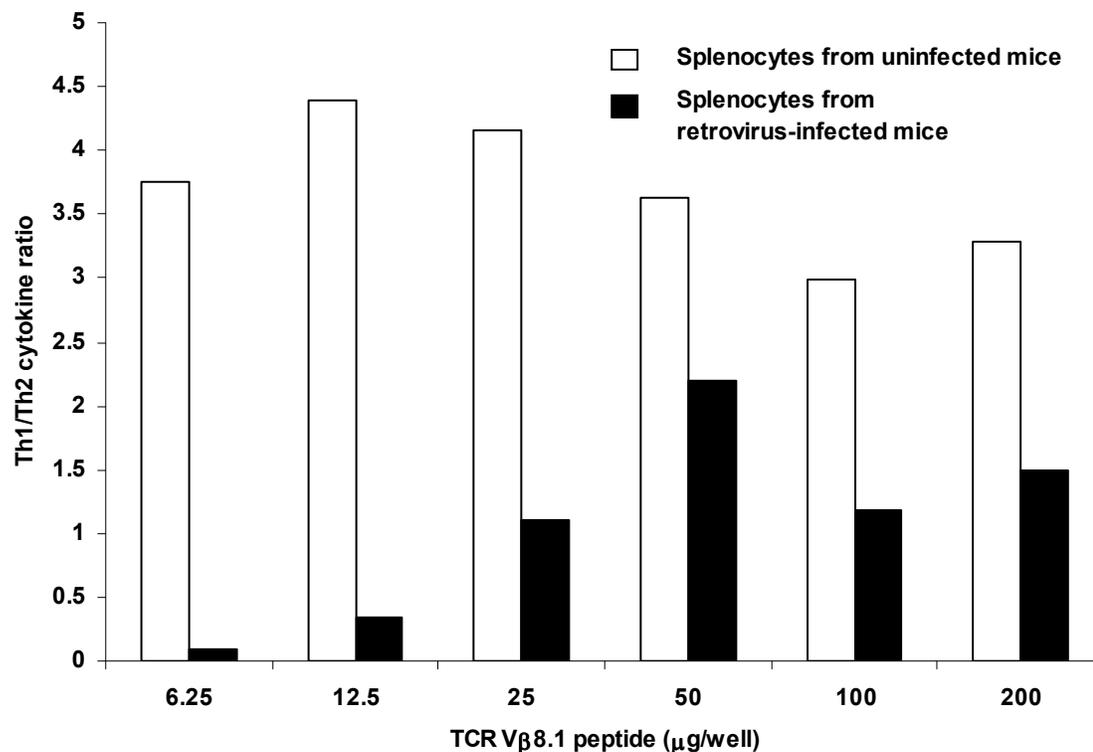
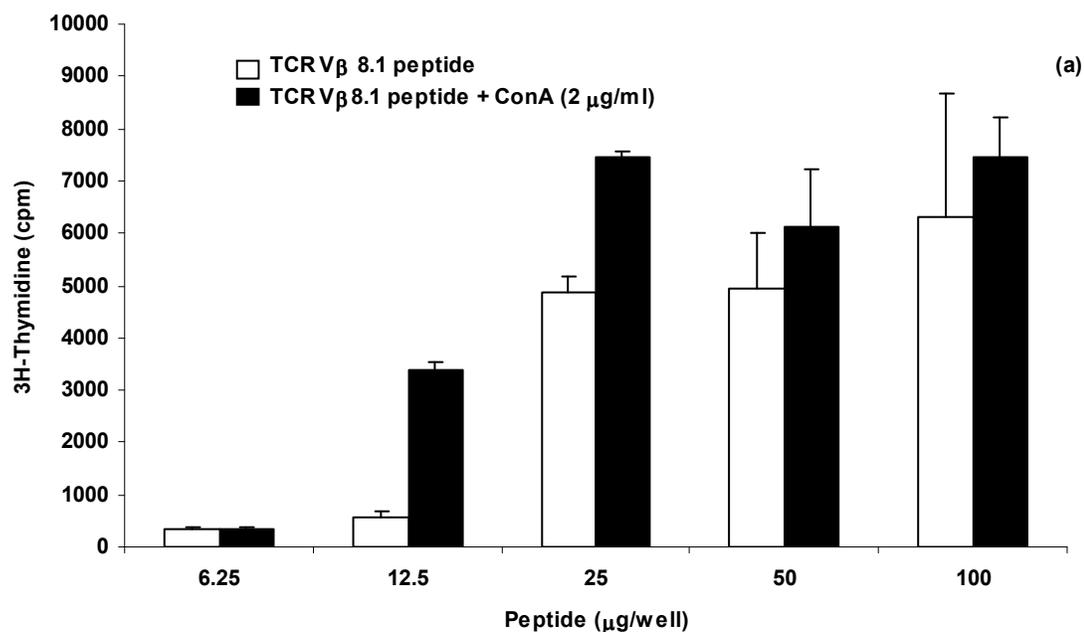


Figure 2.2: Th1/Th2 cytokine ration from non-retrovirus infected and retrovirus infected splenocytes. Ratio was determined based on IL-2 (Th1) and IL-6 (Th2) production by each group. Cytokine production was done by stimulating retrovirus infected murine splenocytes or uninfected splenocytes with different concentrations of TCR Vβ8.1 peptide and Con A (2 μg/mL for 24 h) for IL-2 or LPS (2 μg/mL for 24 h) for IL-6.

2.2.3 Mitogenesis of Splenocytes from Retrovirus Infected Mice

Because peptides related to tumor rejection antigens have showed immunoregulating properties, studies were done to determine whether the TCRVβ8.1 peptide had an effect on lymphocyte mitogenesis in vitro. The increasing amounts of TCR Vβ8.1 peptide showed a titering effect on splenocyte proliferation from retrovirus

infected mice (Figure 2.3). The combination of TCR V β 8.1 peptide + LPS also showed a titering effect with similar readings for the different concentrations of the peptide and constant amount of LPS (Figure 2.3a). The combination of TCR V β 8.1 peptide + Con A yield a higher splenocyte proliferation then the peptide + LPS assay, indicating a cumulative mitogenic effect on T cells (Figure 2.3b). Con A, LPS or β 1 control peptides by themselves did not produce maximum proliferation (Data not shown). Healthy splenocytes obtained from non-retrovirus infected mice and treated with the TCR V β 8.1 peptide showed a similar titering effect but with greater mitogenesis.



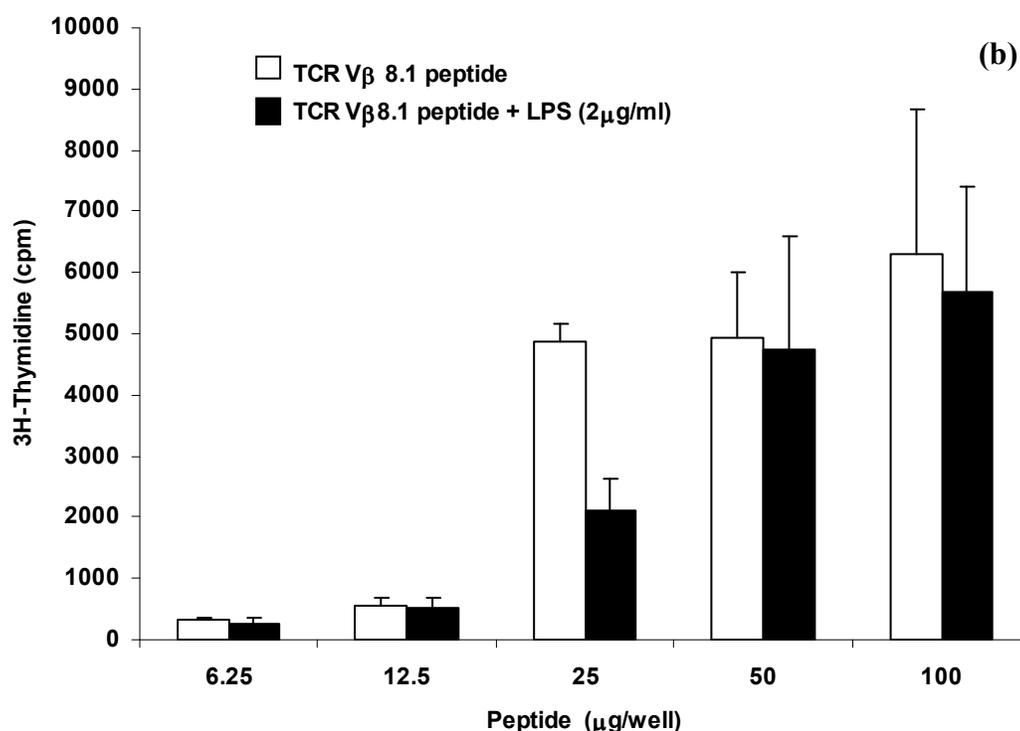


Figure 2.3: The relative proliferation of retrovirus infected splenocytes stimulated by TCRV β 8.1 peptide, with or without LPS (a) or Con A (b). Every sample from Con A or LPS + TCR V β 8.1 peptide treated cells was determined in triplicate. Doses of the TCR V β 8.1 peptide are as indicated in a total volume of 0.1 mL per well. Splenocytes were isolated from LP-BM5-infected C57Bl/6 mice and cultured 1×10^5 cells per well in RPMI 1640 media supplemented with 300 mg/L of L-glutamine, 100 Units/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin. Concentration of Con A and LPS used was 2 $\mu\text{g}/\text{mL}$, which did not produce maximum splenocyte proliferation. Cells were harvested at 48 h of incubation at 37°C and 5% CO₂.

2.2.4 Survival

After 90 days of retrovirus infection, mice were co-infected with CVB3 for 35 days. Of the retrovirus + CVB3 co-infected mice, 45% died within 20 days of CVB3 infection and the remaining 55% died by day 25 post-CVB3 challenge. For the retrovirus infected mice, the decline in the population was less pronounced, although at day 22, 65%

of the population had survived while 100% of the animals had died by day 35. In the retrovirus infected, TCR V β 8.1 peptide treated mice, 10% of the animals died by day 15 post-CVB3 challenge, and the 66% remaining lived for 35 days. Finally, the retrovirus infected + CVB3 co-infected + TCR V β 8.1 peptide treated mice followed a survival trend very similar to that of the retrovirus infected + TCR V β 8.1 peptide treated group, with 55% of the population surviving after 35 days of CVB3 infection (Figure 2.4).

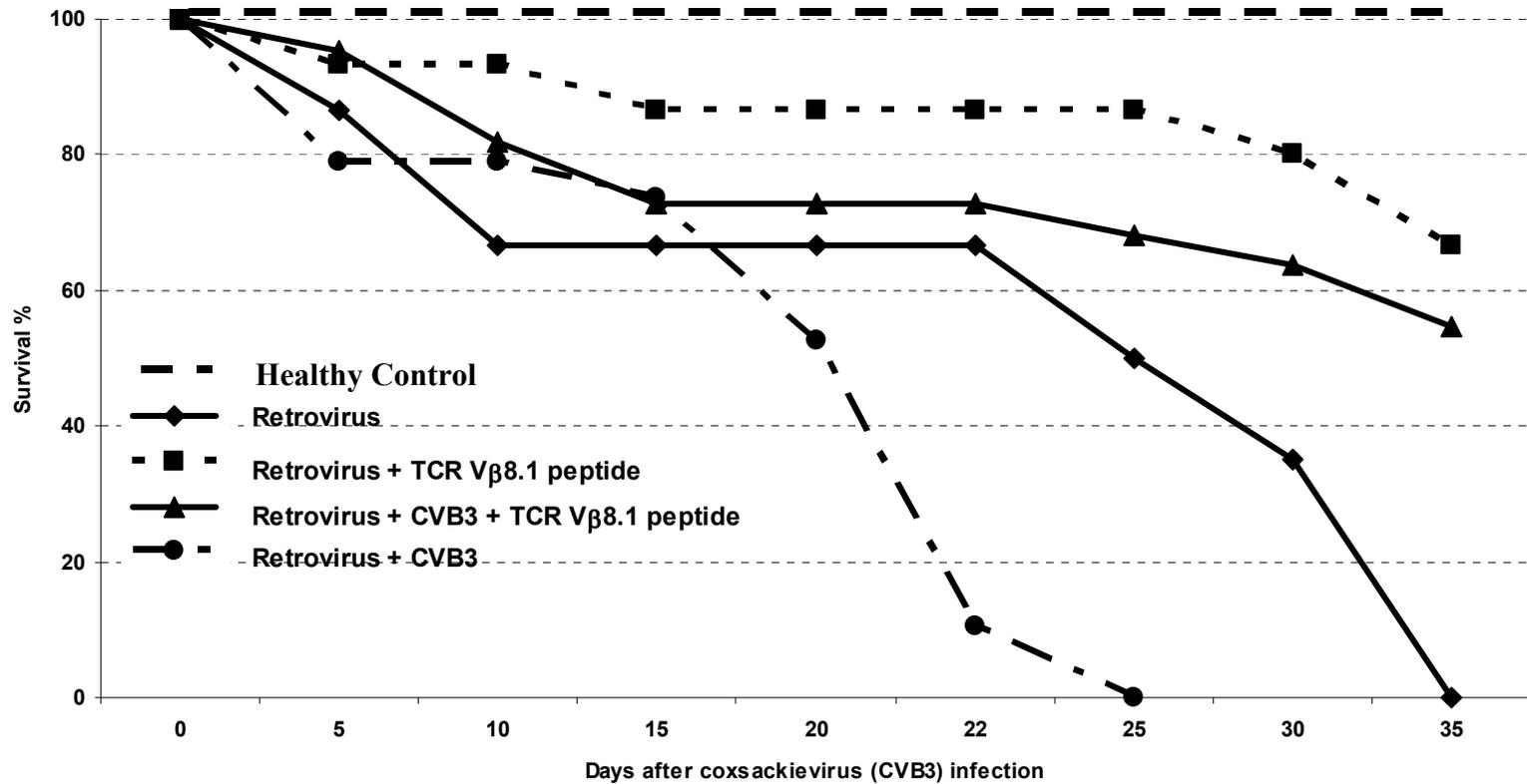


Figure 2.4: Survival curve for retrovirus-infected mice co-infected with CVB3/59 and treated with TCR Vβ8.1 peptide. Day 0 indicates time of CVB3/59 infection. Study ended at 35 days after CVB3 infection. N= 20 mice per group. Non-retroviral infected groups with N=10 mice per group, all survived after 35 days of beginning of the survival study (data not shown).

2.2.5 Histopathology

Healthy C57BL/6 mice do not develop cardiopathology when challenged with CVB3, although they become susceptible when immunodeficient due to LP-BM5 infection. To determine if TCR V β 8.1 treatment would protect retrovirus-infected immunodeficient mice from developing cardiopathology due to CVB3 infection, C57BL/6 mice were infected with LP-BM5 for 3 months and then co-infected with CVB3/59 for 12 days. TCR V β 8.1 or control peptides were treated at 14 and 45 days after retroviral infection. Of the retrovirus infected + CVB3 co-infected mice treated with the control peptide, 87.5% developed cardiopathology with scores 3+ to 4+. However, when identically treated mice were also injected with TCR V β 8.1 peptide, only 35% developed mild cardiopathology with a score of 2+ (Figure 2.5 and Figure 2.6).

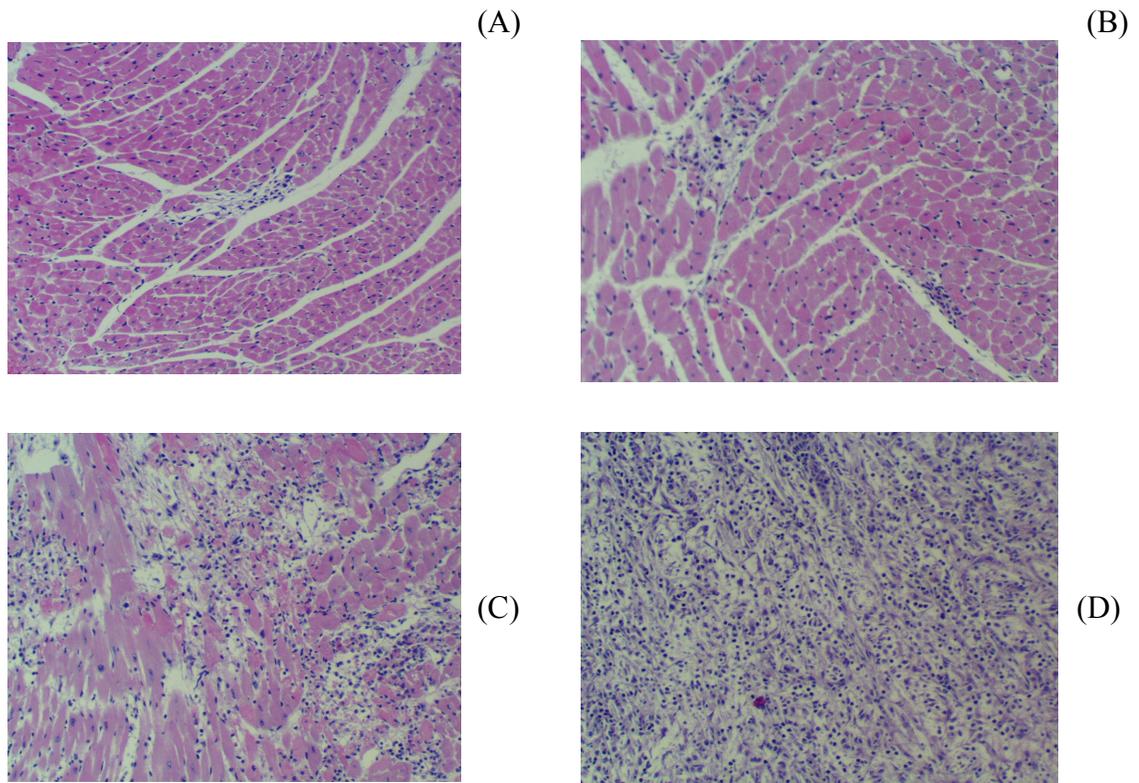


Figure 2.5: Grading of the extent of inflammatory lesions within the myocardium. Staining was with haematoxylin-eosin. Pathology score: 0= no lesions, (A) 1+ = mild multifocal non-suppurative myocarditis, (B) 2+ = mild focal or multifocal non-suppurative myocarditis with myocardocyte degeneration and necrosis, (C) 3+ = moderate focal or multifocal non-suppurative myocarditis with myocardocyte degeneration and necrosis, (D) 4+ = severe multifocal non-suppurative myocarditis with myocardocyte degeneration and necrosis. Mild damage: <10%; moderate damage: 10-25%; severe damage: >25% of heart tissue affected.

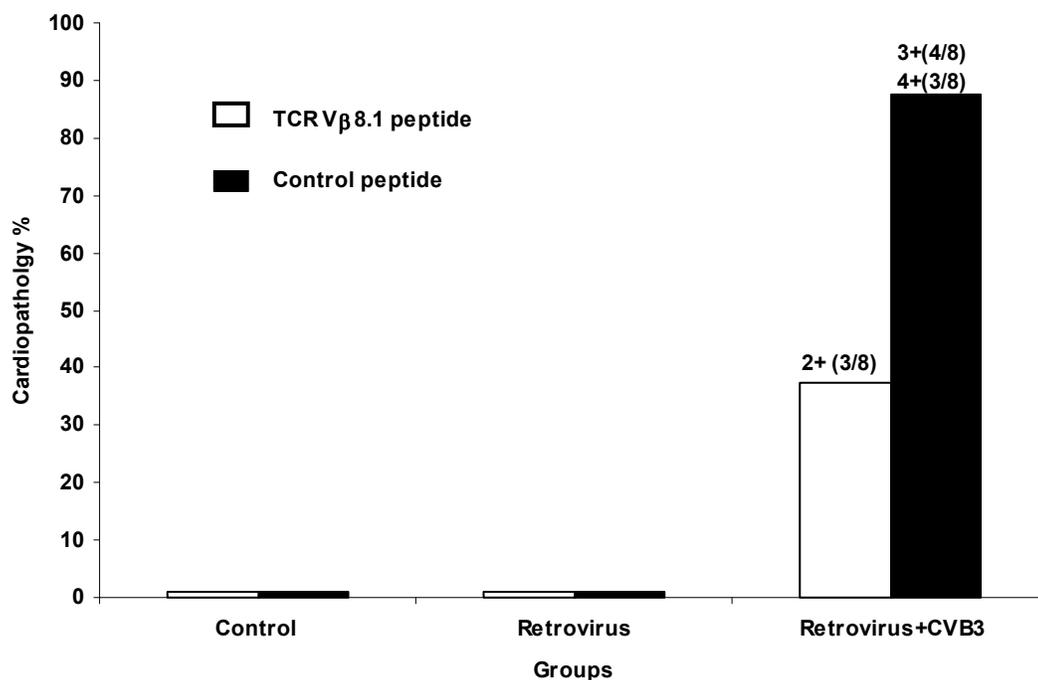


Figure 2.6: Histopathologic scores of Retrovirus-infected mice co-infected with CVB3/59 and treated with TCR Vβ8.1 peptide.

Patients who have human immunodeficiency virus (HIV) infection have low levels of selenium (Se), and Se supplementation is associated with the improvement of T-cell function, reduced apoptosis, and an increase in patient survival. Micronutrient and specifically Se deficiencies accentuate immunodeficiency in AIDS while affecting the pathogenicity of several viruses (Beck et al., 2000). Studies in our laboratory have shown that mice that have AIDS are highly susceptible to CVB3 induced cardiopathology (Sepulveda, et al), and that Se supplementation will induce a decrease in splenomegaly as well as in antibody production (Chen et al., 1997), and increase resistance to CVB3 induced myocarditis (Sepulveda et al., 2003). Based on the studies done by Beck et al., we decided to determine if the same CVB3/0 (amyocarditic) strain of Coxsackievirus B3

would induce cardiopathogenicity in a susceptible strain of mouse (C3H) after one pass in a retrovirally induced immunodeficient host. We determined that CVB3/0, after one pass on the LP-BM5 immunodeficient mice (CVB3/0-LP-BM5+), increased its virulence in the C3H susceptible mice, inducing a degree of cardiopathology not developed in the control group. Studies done in our laboratory, were retrovirus infected mice have been fed a diet supplemented with selenium and then co-infected with CVB3/59 developed only mild myocarditis and increase survival as opposed to the equally infected mice with no selenium supplementation (Sepulveda et al., 2002c). We concluded that, as well as in the Se deficient studies, that an immunodeficient host also has the conditions to allow a phenotypical change of virulence of CVB3/0.

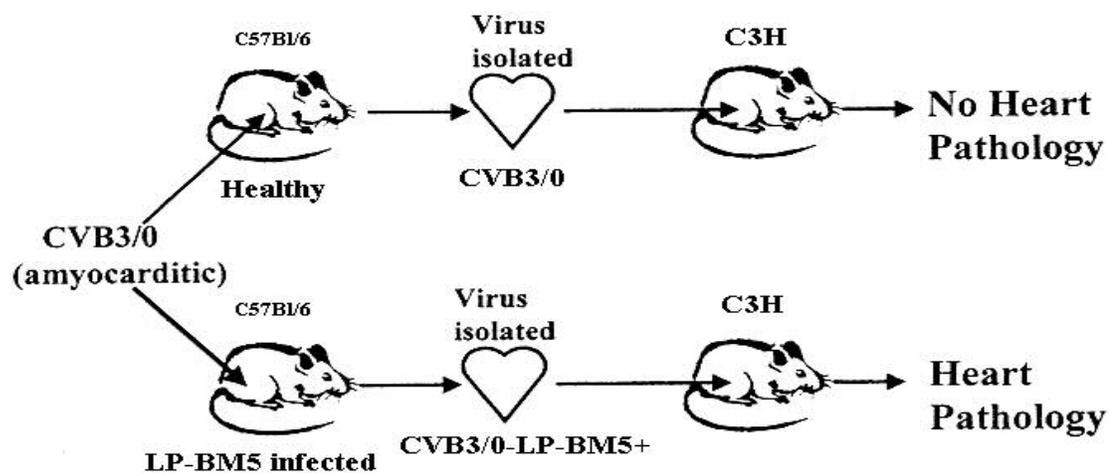


Figure 2.7: Diagram depicting virus passage experiment and outcome. Mice were infected with LP-BM5 retrovirus which makes them Se-deficient. After 3 months of retroviral infection, mice were inoculated with amyocarditic coxsackievirus strain CVB3/0. Seven days after infection, virus was isolated from the mice (renamed CVB3/0-LP-BM5+) and passed back into healthy mice. Only the CVB3/0-LP-BM5+ virus induced myocarditis in healthy (Se-adequate) mice.

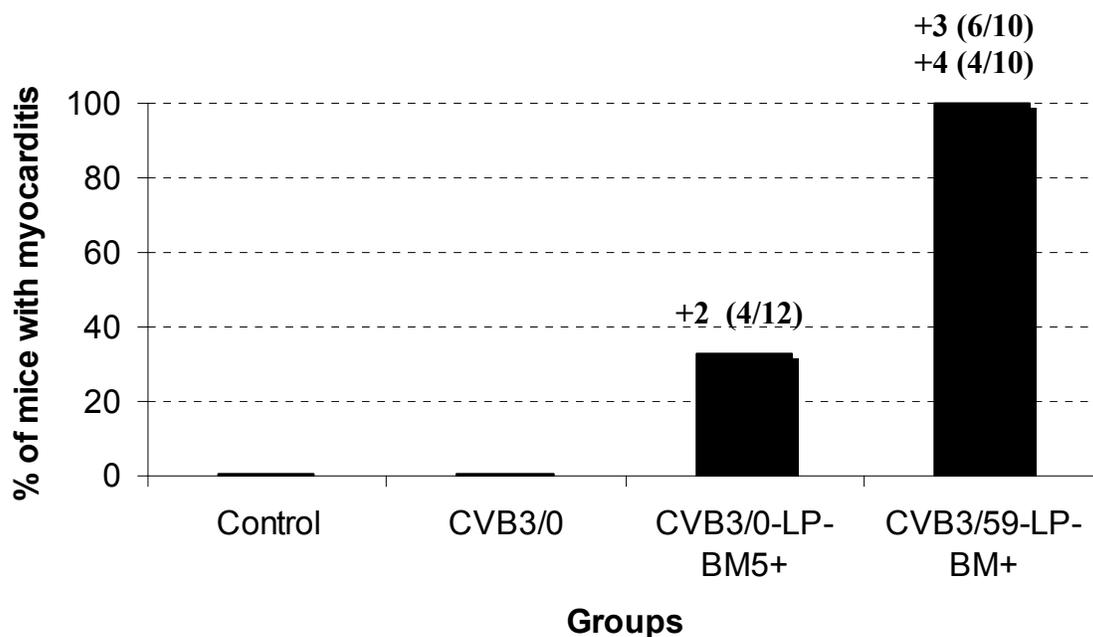


Figure 2.8: Cardiopathology of virus passage experiment. 12 mice per group.

2.3 Discussion

When immunodeficient mice were treated with the TCR V β 8.1 peptide and co-infected with CVB3, only 38% developed a mild form of cardiopathology while in the same non-peptide treated group 89% showed significant and severe cardiac lesions. With the immunodeficiencies caused by the LP-BM5 retrovirus and HIV, the Th1 response is decreased, debilitating the immune response and the clearance of viral infections. CVB3 infections are being widely recognized as one of the major pathogens causing cardiopathology in immunodeficient patients. Studies in our laboratory have shown that CVB3 co-infection of mice with murine AIDS develop severe myocarditis, while healthy (non-retrovirus infected) mice are resistant. Although peptide treatment does not entirely prevent cardiopathology, treated populations show a marked decrease in cardiac lesion

severity. This finding may offer the possibility of a greater chance of viral clearance as well as enhanced quality of life. When retrovirus infected mice were treated with the TCR peptide and then co-infected with CVB3, the survival rate was greater as compared to the same group not treated with the peptide. In our study more than 50% of the treated populations were still alive at day 35, while none of the non-treated animals survived beyond day 25. Our results strongly suggest that the peptide provides a protective effect against the development of myocarditis due to the coxsackieviral infection as reflected in the extended survival of the treated mice. The results also show that when retrovirus-infected mice are treated with the TCR peptide, their survival rates improve. Murine retroviral infection did not cause myocarditis but is characterized by the presence of lymphomas due to the retroviral infection of B cells. Viral infections and cancer cells are best combated by the T_H1 arm of the immune system, since the TCR peptide promotes IL-2 production, a Th1 cytokine. Consequentially, there will also be CTL and NK cell activation important in the clearance of cancer cells. Although the TCR peptide therapy does not cure the mice of murine AIDS, it does slow the progression of the retroviral infection, as observed by the smaller size of the lymph nodes and spleen and as a consequence, extends the lifespan.

The TCR peptide in vitro and in vivo protected Th1 responses from retroviral damage. T_H1 protective effects may be important to prevent cardiopathology caused by CVB3 infection. When splenocytes from retrovirus infected mice were treated in vitro with increasing amounts of TCR V β 8.1 peptide, IL-2 production increased as a measure of Th1 cell activity. However, the addition of Concanavalin A, a T-cell mitogen, had no

effect on the production of IL-2. Uninfected splenocytes maintained an average Th1/Th2 cytokine ratio of 3.5 with increasing amounts of the peptide. With the splenocytes from retrovirus infected mice, the TCR V β 8.1 treatment showed an increase in the ratio as the concentration of the peptide also increased, showing a peak of 2 with a concentration of 50 μ g/well of TCR V β 8.1 peptide. The TCR V β 8.1 peptide increases production of IL-2, inducing a more balanced Th1/Th2 response for a more effective cell mediated response needed for the clearance of the retroviral infection as well as any secondary viral infections that might occur. These changes could explain the greater survival in the TCR peptide treated mice even though the viral burden was not measured.

At certain concentrations, the TCR V β 8.1 peptide induces splenocyte proliferation. Splenocytes treated with peptide and LPS, a B cell mitogen, showed no added proliferation. However, splenocytes treated with peptide plus Con A, a T-cell mitogen, gave an accentuated proliferative effect as indicated with tritiated thymidine incorporation. The fact that higher concentrations of TCR V β 8.1 peptide induces IL-2 production with a subsequent increase in the Th1/Th2 cytokine ratio and that Con A will increase the mitogenic effect of the peptide suggests that the TCR V β 8.1 peptide targets T cells.

In conclusion, our results indicate an important immunoregulatory effect of the TCR V β 8.1 peptide in murine AIDS. The TCR V β 8.1 peptide restored Th1 activity which protects against the development of cardiopathology caused by CVB3 infection. The TCR V β 8.1 peptide also extended the lifespan of both mice with AIDS and mice superinfected with CVB3. The peptide also encourages a greater Th1 response through IL-2

production, which may help reduce viral load. The actions of the TCR V β 8.1 peptide may not only aid us in our understanding of HIV mechanisms, but they may also provide ideas of novel therapies to help prolong the quality and quantity of life for those patients suffering with HIV.

CHAPTER 3

T-CELL RECEPTOR V β 8.1 PEPTIDE REDUCES COXSACKIEVIRUS INDUCED CARDIOPATHOLOGY IN AGED MICE

Immunosenescence is characterized by reduced levels of the peripheral naïve T-cell pool derived from thymus and the loss of immature B lineage cells in the bone marrow. In humans as well as in animals disease resistance declines in direct proportion to aging. Although both the innate and adaptive arms of the immune systems are changed, the adaptive compartment is most affected. Innate immunity is established immediately after birth and changes only slightly throughout life. However, adaptive immunity is immature in neonates and optimal in young adults and progressively declines thereafter. In elderly humans naïve T, total B cells, and CD5+ B lymphocytes are decreased whereas activated and memory T cells, as well as natural killer (NK) cells are expanded. Aged mice show decreased numbers of Thy1+ cells in the thymus, a higher number of cells expressing IL-2R, and a lower number of IgA+ plasma cells in the internal lamina propria than do young mice. In old mice, T- and B-cell proliferation is reduced. In addition, TNF- α , IL-4, and IL-6 are secreted more abundantly, whereas there is a decrease in IL-2.

The objective of this study was to determine if TCR V β 8.1 peptide would promote immunoregulation sufficient to resist the development of cardiopathology from CVB3 infection in aged mice.

3.1 Materials and Methods

3.1.1 Animals

Female C57BL/6 mice, 17 months of age (old mice) from the Charles River Laboratories Inc. (Wilmington, DE) were cared for as required by the University of Arizona Committee on Animal Research. After 2 weeks housing in the Central Animal Facility, University of Arizona, they were randomly assigned to one of the following treatments with 12 mice per group: (1) uninfected normal mice injected with MCG3 (a synthetic peptide containing the CDR1 sequence of MCG- λ L chain) control peptide, (2) uninfected normal mice injected with MCG3 and infected with CVB3, (3) uninfected normal mice injected with TCR V β 8.1 peptide, (4) uninfected normal mice injected with TCRV β 8.1 and infected with CVB3. Administration of peptides (200 μ g/mice in saline, i.p.) was performed twice, at 17.5 months and 18 months. At 18 months, the appropriate groups of mice were infected with CVB3.

3.1.2 Peptides

A set of overlapping 16-mer peptides that duplicate the covalent structure of the VBDBJBC β protein predicted from a human TCR-V β sequence was used (Marchalonis et al., 2001b). Here we focus on the sequence C K P I S G H N S L F W Y R Q T that corresponds to the complete CDR1 and N-terminal five residues of Fr2 of the human V β 8.1 gene product as described earlier (Marchalonis et al., 2001b). As a control peptide we used the following peptide sequence T G T S S D V G G Y N Y V S W Y.

3.1.3 Coxsackievirus B3 infection

Cardiovirulent coxsackievirus B3 serotype 59 stocks were propagated and titered as described earlier. After 3 months of retroviral infection, mice were inoculated intraperitoneally with 3×10^5 TCID₅₀ of CVB3 strain 59 in 0.1 ml of MEM. Mice were sacrificed after 12 days of CVB3 infection.

3.1.4 Histopathology

At indicated times after inoculation, mice were killed and their hearts removed for study ($n= 12$ heart samples per group). Hearts were rinsed in saline and transversely cut in half. One half of each heart was immediately placed into Histochoice Tissue Fixative (AMRESCO, Solon, OH, U.S.A.) and stored at room temperature. Fixated hearts were sectioned (6 μ m) on a Zeiss HM 505 N cryostat (Carl Zeiss, Thornwood, NY, U.S.A.) and stained with hematoxylin and eosin. The severity of inflammatory lesions within the myocardium was graded by a pathologist without knowledge of the other experimental variables. Grading was performed in a semiquantitative manner according to the relative degree (from heart to heart) of mononuclear cell infiltration and the extent of necrosis. Mild damage is considered as <10% of heart tissue affected, moderate = 10-25% and severe as >25% of heart tissue affected.

3.1.5 Heart viral titers.

At the indicated times after inoculation, mice were sacrificed and one-half of the heart removed for viral titers. Heart halves were rinsed in saline and immediately frozen on dry ice and then stored at -80°C until processed. Heart sections were weighed, then ground in a small volume of RPMI-1640 using a Ten Broeck homogenizer (Fisher Scientific) and freeze-thawed three times. The ground tissue was then centrifuged (2000 x g), and the resulting supernatant was recovered for assay. Supernatant was titered on HeLa cell monolayers by TCID₅₀ using ten fold dilutions (Sepulveda et al., 2002).

3.1.6 ELISA for Cytokines

The production of interleukin (IL)-2, and IL-6 from mitogen stimulated splenocytes was determined as described previously. The cytokines were determined by sandwich ELISA using a commercial Endogen kit (Woburn, MA, U.S.A.), as described in previous studies (Sepulveda et al., 2002a,b).

3.1.7 Statistics

All variables were compared using one-way analysis of variance (ANOVA) for comparison between any two groups. Differences between two groups were considered significant at $p < 0.05$.

3.2 Results

3.2.1 Histopathology

Young and mature-healthy C57BL/6 mice (3-10 month old) do not develop cardiopathology when challenged with CVB3 although it has been previously shown that they become susceptible when immunodeficient due to retrovirus infection and its attendant cytokine dysregulation. To determine whether TCR V β 8.1 treatment would protect aged mice from developing cardiopathology due to CVB3 infection, C57BL/6 mice were infected with CVB3 at 18 months of age for 12 days. TCR V β 8.1 or control peptides were administered at 2 weeks and on the day of CVB3 infection. Aged mice infected with CVB3 and treated with the control peptide developed cardiopathology with scores 1+ (25%), 2+ (50%) and 3+ (25%). However, when identically infected mice were treated with TCR V β 8.1 peptide, 18% of the group showed 1+ scores, 10% showed 2+ and only 9% showed a 3+ score, while 62% of them presented no pathology (Figure 3.1).

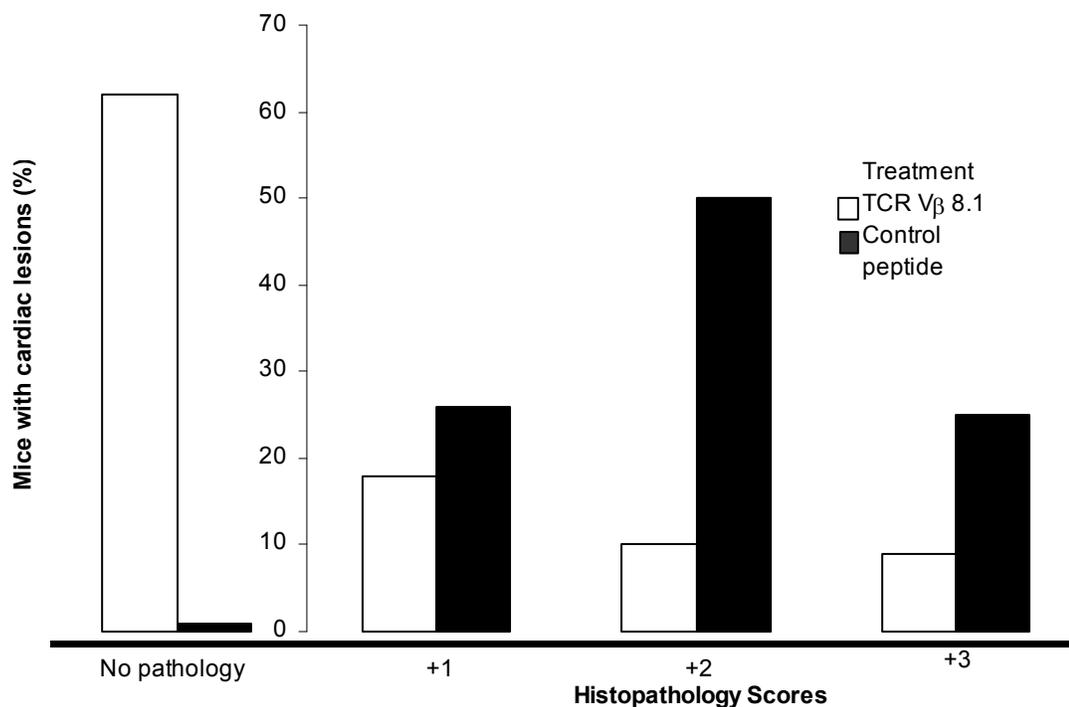


Figure 3.1: Histopathologic scores of aged-mice infected with CVB3/59 and treated with TCR V β 8.1 peptide. $N = 12$ heart samples per group. Pathologic score: 0, no lesions; 1+, mild multifocal non-suppurative epicarditis to mild multifocal non-suppurative myocarditis; 2+, mild focal to multifocal non-suppurative myocarditis with myocardiocyte degeneration and necrosis; 3+, moderate focal to multifocal non-suppurative myocarditis with myocardiocyte degeneration and necrosis; 4+, severe multifocal non-suppurative myocarditis with myocardiocyte degeneration and necrosis. Mild damage is considered as <10% of heart tissue affected, moderate = 10-25% and severe as >25% of heart tissue affected.

3.2.2 Viral titers

To determine whether the difference in cardiopathology between aged mice infected with CVB3 + control peptide and aged mice infected with CVB3 + TCR V β 8.1 peptide was due to a difference in the viral titer, the geometric mean titers of virus recovered from the hearts of infected animals were determined. Hearts from individual mice infected with CVB3 were assayed for the presence of replicating virus in the tissue

(Table 3.1). Hearts from aged mice infected with CVB3 and treated with the control peptide had increased viral titers after 12 days of infection than aged mice similarly infected and treated with the TCR V β 8.1 peptide ($p < 0.05$). All control and aged mice injected with control peptide and not challenged with CVB3 as well as aged mice treated with the TCR V β 8.1 peptide showed no signs of pathology.

Table 3.1: Cardiac viral titers of CVB3/59 from heart tissue of aged mice treated with TCR V β 8.1 peptide.

Treatment			Cardiac viral titer ^a
Control Peptide	TCR V β 8.1 peptide	CVB3/59	TCID ₅₀ /g tissue
+	-	-	ND
-	+	-	ND
+	-	+	1.0×10^8 ($2.8 \times 10^7 - 5.4 \times 10^8$)
-	+	+	2.5×10^7 ($5.1 \times 10^6 - 7.5 \times 10^7$) ^b

^a Heart viral titers are expressed as means of 8 to 10 mice per group, with the range in parentheses.

^b Significantly different to the control group ($p < 0.05$)

ND= Not Detectable

3.2.3 Cytokine Production by Splenocytes

To determine the effect on cytokine production of TCR V β 8.1 peptide on aged mice infected with CVB3, Th1 (IL-2, TNF- α and IFN- γ), Th2 (IL-4) and IL-6 cytokine production was assessed (Tables 3.2 and 3.3). Production of IFN- γ by Con A-stimulated splenocytes was significantly ($p < 0.05$) inhibited in aged mice infected with CVB3 when compared to the non-CVB3 infected group. TCR V β 8.1 peptide treatment significantly

increased IFN- γ (2 fold) production in the CVB3 infected group when compared to the control peptide treated animals ($p < 0.05$). For the same groups, TNF- α showed significant change ($p < 0.05$), Table 3.2.

Table 3.2: Effect of TCR V β 8.1 peptide on Th1 cytokine production in old mice infected with coxsackievirus B3.

Groups			Cytokines (ng/ml)		
Control Peptide	TCR V β 8.1 peptide	CVB3/59	IL-2	IFN- γ	TNF- α
+	-	-	0.43 \pm 0.15	16.8 \pm 1.4	1.45 \pm 0.1
-	+	-	0.95 \pm 0.1	26.4 \pm 1.71	0.75 \pm 0.13
+	-	+	1.16 \pm 0.13	10.7 \pm 0.5	0.85 \pm 0.2
-	+	+	1.75 \pm 0.2	20.2 \pm 0.3	1.2 \pm 0.1

Splenocytes (1×10^7 cells/ml in RPMI medium) were incubated with Con A or LPS at 37°C. After collection of the supernatants, cytokines were determined using a Pirce-Endogen KM-minikit. Each sample ($n = 12$ per group) was tested in triplicate. The concentration of the cytokines were determined by ELISA at 450 nm. Data is presented as means (95 CI) of triplicate wells.

Both the humoral immune response cytokines determined (IL-4 and IL-6) showed a significant decrease in their production in the TCR V β 8.1 peptide treated group infected with CVB3 when compared to the control peptide treated animals ($p < 0.05$).

Table 3.3: Effect of TCR V β 8.1 peptide on a Th2 and IL-6 cytokine production of old mice infected with coxsackievirus B3.

Groups			Cytokines (ng/ml)	
Control Peptide	TCR V β 8.1 peptide	CVB3/59	IL-4	IL-6
+	-	-	0.49 \pm 0.12	1.81 \pm 0.21
-	+	-	0.23 \pm 0.21	1.25 \pm 0.22
+	-	+	1.15 \pm 0.1	1.90 \pm 0.16
-	+	+	0.75 \pm 0.05	1.05 \pm 0.1

Splenocytes (1×10^7 cells/ml in RPMI medium) were incubated with Con A or LPS at 37°C. After collection of the supernatants, cytokines were determined using a Pirce-Endogen KM-minikit. Each sample ($n=12$ per group) was tested in triplicate. The concentrations of the cytokines were determined by ELISA at 450 nm. Data is presented as means (95 CI) of triplicate wells.

The TCR V β 8.1 peptide increases production of Th1 cytokines, inducing a more balanced Th1/Th2 response for more effective cell-mediated responses for the clearance of coxsackievirus infection (Figure 3.2).

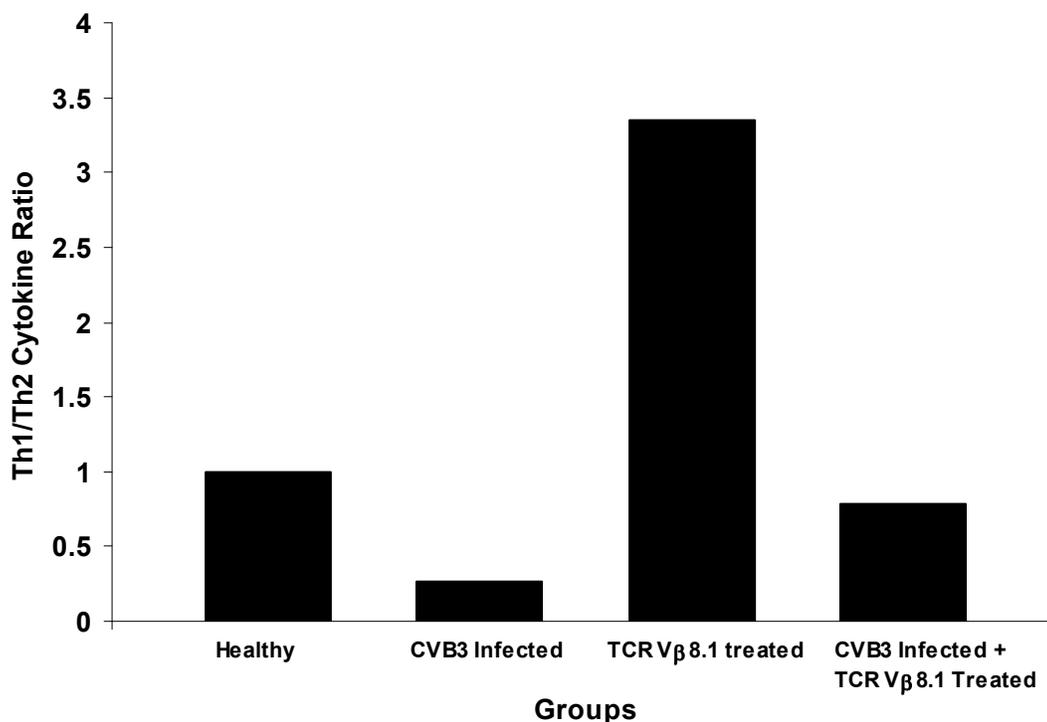


Figure 3.2: Cytokine ratio Th1/Th2 from splenocytes of coxsackievirus infected mice treated with TCR Vβ 8.1 peptide. Ratio was determined based on interferon gamma (IFN- γ) (Th1) and interleukin-4 (IL-4) (Th2) production by each group. Cytokine production was done by stimulating coxsackievirus infected splenocytes or uninfected splenocytes with different concentrations of T-cell receptor (TCR) Vβ8.1 peptide and concanavalin A (Con A) (2 μ g/ml for 48 h) for both cytokines.

3.3 Discussion

In animals as well as in humans, aging reduces the competence of the immune system to maintain the hosts integrity against pathogens as well as to mount an efficient response against cancer cells. Our results show that cytokine dysregulation due to immunosenescence suppresses Th1 cytokine production. TCR Vβ8.1 peptide therapy stimulated Th1 cytokines sufficiently enough to decrease the development of myocarditis

due to a cardiovirulent pathogen. Of the three Th1 cytokines determined, IL-2 and IFN- γ were suppressed the most in the infected non-TCR V β 8.1 treated group. TCR V β 8.1 peptide administration to aged mice rapidly increased IL-2 cytokine production two fold, compared to the control peptide treated animals (Table 3.2). CVB3 infection by itself increased IL-2 production considerably when measured in control peptide treated animals, and TCR V β 8.1 peptide administration for the same treatment group enhanced IL-2 production (Table 3.2). IFN- γ production was suppressed after CVB3 infection in the control group. However, treatment with the TCR V β 8.1 peptide in the CVB3 and non-CVB3 infected animals increased IFN- γ production above the non-treated non-infected group (Table 3.2 and 3.3). Moreover, IFN- γ production by aged mice after TCR V β 8.1 peptide treatment, especially in the non-CVB3 infected group, showed levels similar to those seen in young 14 week old mice (Colombo et al., 1999; Liang et al., 1998; Watson et al., 1995; Marchalonis et al., 2001; Sepulveda et al., 2002a; Sepulveda et al., 2000; Sepulveda et al., 2002b). The production of both IL-2 and IFN- γ after TCR V β 8.1 peptide treatment in aged mice inversely correlates with the frequency and severity of cardiopathology due to CVB3 infection (Figure 3.1) as well as with the presence of viral particles from heart muscle tissue (Table 3.1). In Figure 2, we determine the ratio of the Th1/Th2 cytokine response based on IFN- γ and IL-4 production. The TCR V β 8.1 peptide treatment increases production of Th1 cytokines, inducing a more balanced Th1/Th2 response. A higher ratio produces more efficient cell-

mediated responses for the clearance of coxsackievirus infection as shown by the viral titers determined in heart tissue of peptide treated mice (Figure 3.2 and Table 3.1).

The cardiopathology caused by coxsackivirus occurs via two mechanisms. The virus infects myocytes to induce cardiopathology. There is also an autoimmune aspect of infection where the virus induces antibodies directed against both itself and against heart autoantigens in the host (Henke et al., 2001; Badorff et al., 2000; Beisel et al., 1991). The finding of decreased pathology in addition to the decrease in viral titers in the heart tissue of mice treated with peptide indicates that the peptide influences both pathways leading to a decrease in viral induced cardiopathology. Peptide treatment promotes a more balanced Th1/Th2 cytokine ratio and therefore a more efficient cellular immune response. This occurs because the peptide increases Th1 cytokine production while decreasing Th2 levels as compared to controls (Tables 3.2 and 3.3). The decreased Th2 cytokine production may influence a decrease in the antibody production leading to less severe cardiopathology.

In conclusion, our results show an important immunoregulating effect of TCR V β 8.1 peptide in aged mice. The TCR V β 8.1 peptide induced Th1 cytokine production that promotes a more capable cellular immune response which in turn protects the host against the development of myocarditis due to CVB3 infection. TCR V β 8.1 peptide also promoted the clearance of CVB3 viral particles from heart muscle tissue. This study further supports the potential of TCR V β 8.1 peptide therapy in host immunostimulation as well as in immunoregulation against viral and autoimmune diseases.

CHAPTER 4

IMMUNE ENHANCING EFFECT OF THE NOVEL TCR V β 8.1 PEPTIDE IN JURKAT CELLS

Treatment with TCR V β 5.2 and -V β 8.1 peptides alone and combined largely prevents the development of retroviral induced immunodeficiency in mice. Also, TCR V β 8.1 peptide therapy in the immunodeficient mice protects them from the development of viral myocarditis induced by a secondary infection. Studies with these peptides show that they possess an immunoregulatory effect enhancing Th1 cytokine production while stabilizing Th2. The studies to date do not establish clearly if the peptide requires any processing or presentation by antigen presenting cells (APC's). Here we develop a pilot study to show the effect of the TCR V β 5.2 and -V β 8.1 peptides in a T cell line (Jurkat cells). The peptides induced changes in T cell proliferation as well as in cytokine production. TCR V β 8.1 induced cell proliferation, while TCR V β 5.2 peptide showed inhibition. Both peptides also influenced cytokines production by Jurkat cells. This pilot study showed that both TCR V β 5.2 and -V β 8.1 peptides act directly upon the cell influencing its biological functions.

4.1 Materials and Methods

4.1.1. Cell line

CD4+ Jurkat human leukemia T cells (ATCC TIB-152) were cultured in RPMI 1640 medium (Gibco, Life Technologies, Switzerland) supplemented with 10% fetal bovine serum, 1 μ g/mL fungizone (amphotericin B), 100 units/mL penicillin, 100 μ g/ml

streptomycin, and 2 mM L-glutamine (all from Gibco, Life Technologies) at 37°C and 5% CO₂ in 50 mL culture flasks (TPP). 2 x 10⁶ cells were incubated with 1 mL of fresh medium in a 24-well plate and left to rest for 48 h. The peptides (TCR Vβ5.2 and TCR Vβ8.1) were incubated at 2 μg/100 μl concentration.

4.1.2 Peptide

A set of overlapping 16-mer peptides that duplicate covalent structure of the VBDBJBCβ protein predicted from a human TCR-Vβ sequence has been produced. Here, we focus on the sequence CKPISGHNSLFWYRQT and CSPKSGHDTVSWYQQA, which correspond to the CDR1 segment of the TCR (Vβ8.1 and Vβ5.2 gene products, respectively).

4.1.3 Mitogenesis

2x10⁶ Jurkat cells were cultured in complete media with 2 μg of the corresponding peptides. After 48h incubation, cells were harvested and pulsed with 3H-thymidine for 4h to measure DNA synthesis as a correlation factor for proliferation. Cell counts in a hemocytometer were done to confirm proliferation.

4.1.4 Cytokine protein array

The array is used to accurately identify expression profiles of multiple cytokines. The array determines simultaneously 23 different cytokines. 20x10⁶ Jurkat cells were suspended in 300 μl of media and treated with either ConA (5 μg/100 μl), TCR Vβ5.2 peptide or TCR Vβ8.1 peptide (2 μg/100 μl). Cells were incubated at 35°C and 5% CO₂

for 48 h and supernatant was collected to be use in human cytokine protein array system I (RayBiotech, Inc). In short, human cytokine array membranes were incubated with 1 ml of conditioned media for 2 h. After removing unbound materials, the membranes were incubated with a mixture of biotin labeled antibodies and control (RPMI 1640 medium with 10% FBS and 1% antibiotics). Signals were detected with HRP conjugated streptavidin and ECL. The membrane was exposed to X-ray film for 5h.

4.1.5 Densitometry

The intensities of signals were scanned and quantitated by densitometry (Bio-Rad, Hercules, CA).

4.1.6. cDNA Microarray Analysis

cDNA microarray analysis was done at the Microarray Core Facility at the Arizona Cancer Center in the University of Arizona. From a similar Jurkat cell culture as indicated above, cells were treated with TCR V β 8.1 peptide with (2 μ g/100 μ l of media). Total RNA was isolated from the Jurkat cell culture using the Trizol method. The following protocol was used to isolate total RNA from non-treated Jurkat cells (Cy3) and peptide treated Jurkat cells (Cy5). Fluorescent first strand cDNA from the cultures was made from 20 μ g of total RNA using the Micromax Direct cDNA Microarray System. Each Cy5 labeled cDNA reaction was combined with one of the Cy3 labeled cDNAs using a PCR Purification Kit. After elution from the purification column, the probe was lyophilized to dryness, and resuspended in 15 μ l hybridization buffer, denatured by

boiling for 2.5 min, and added to a microarray. Co-hybridization of the mixture was done on purified PCR products of human cDNA inserts robotically spotted onto chemically activated glass microscope slides. The array was placed in a hybridization chamber at 62°C for 18 h. Slides were scanned for Cy3 and Cy5 fluorescence using an Axon GenePix 4000 microarray reader and quantitated using GenePix software. Gene expression results were analyzed using GeneSpring. Genes that showed at least a 1.5-fold induction or a 0.5-fold reduction in expression were considered biologically important.

4.2 Results

During the early days of TCR signaling research, immunologists had the use of a range of mouse and human T-cell lines capable of mounting biologically relevant responses to TCR stimulation. Prominent members of this legendary group were transformed T-cell lines of human (for example, HPB-ALL, HuT-78) and mouse (EL4, LBRM-33) origins. Although major advances stemmed from the use of each of these cell lines, arguably the most popular and historically significant of this group was the human leukemia T-cell line, Jurkat.

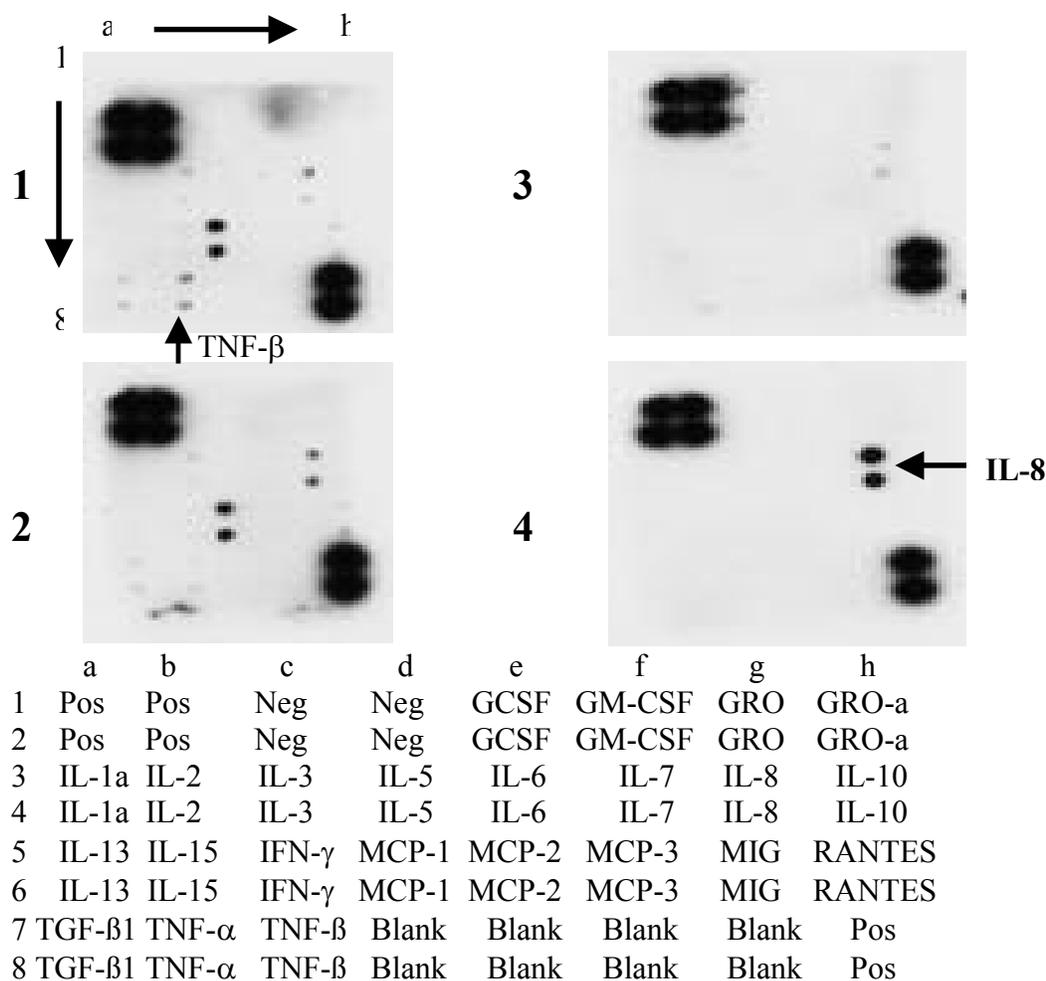


Figure 4.1: Representation of cytokine arrays from conditioned media of Jurkat cell cultures. (1) Control, (2) ConA, (3) TCR V β 5.2 and (4) TCR V β 8.1. Human cytokine array membranes were exposed to X-ray film for 5 h.

As indicated in Figures 4.2 and 4.3, untreated Jurkat cells showed a higher production of most cytokines in the array. When ConA was added cytokine production was decreased and lower cytokine secretion was seen with both of the TCR V β peptides. One cytokine was produced in great amounts (IL-8) in the Jurkat cell culture treated with the TCR V β 8.1 peptide. The intensity levels for IL-8 were not included in the graph

because of dwarfing of other results. Figures 4.2 and 4.3 show increased production of IL-1, IL-2 and TNF- β in the non-treated cultures, typical of Jurkat cell cytokine production.

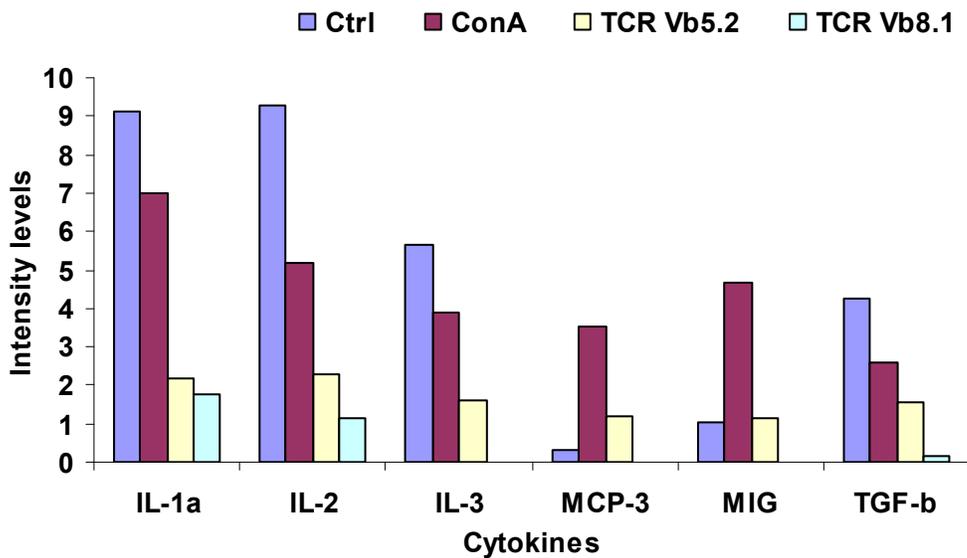


Figure 4.2: Jurkat cell cytokine production by peptide treatment. The relative expression levels of cytokines were determined by densitometry.

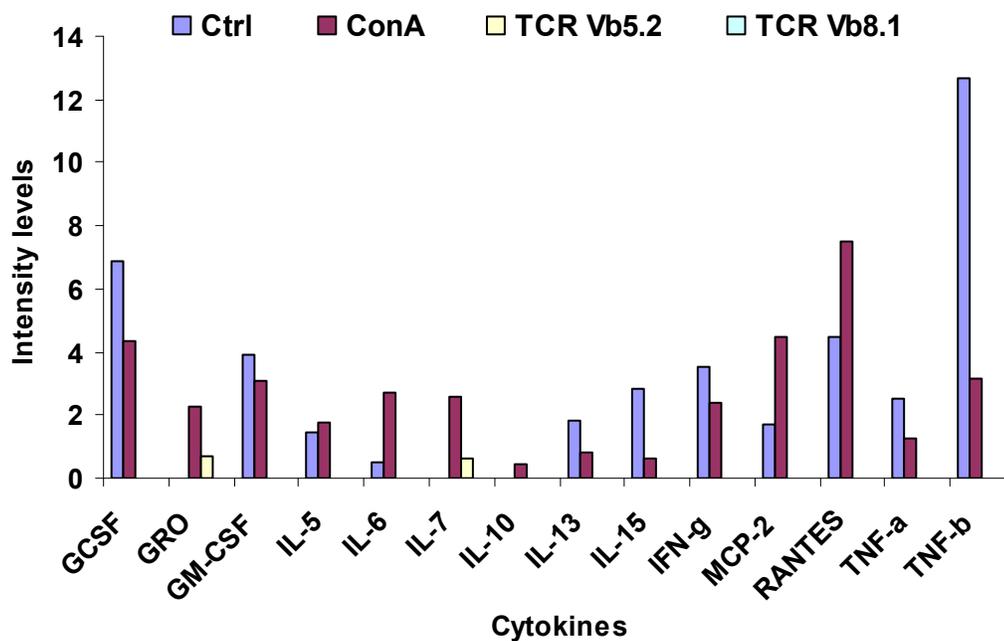


Figure 4.3: Jurkat cell cytokine production by peptide treatment. The relative expression levels of cytokines were determined by densitometry.

When Jurkat cell proliferation was assessed with addition of the different stimuli to the cultures, TCR V β 8.1-peptide treated Jurkat cells showed increased thymidine incorporation. This apparent increase in proliferation could be interpreted as follows: thymidine incorporation to determine cell proliferation has limitations in the sense that cells that synthesize DNA do not necessarily divide or some of the incorporation may be indirect. One possible explanation might be that thymidine incorporation was increased

due to DNA repair. One way to clarify this dichotomy will be to do a direct cell count to correlate with ^3H -thymidine counts per minute (cpm), Figure 4.4.

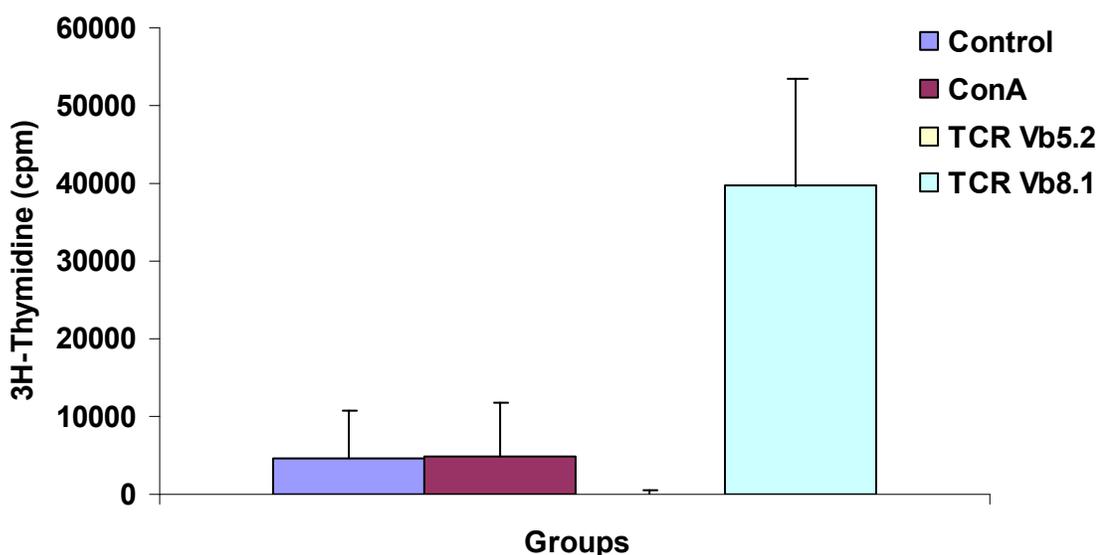


Figure 4.4: Jurkat cell induced proliferation with immunoregulatory peptides. 8 samples per group.

As indicated above, non-treated Jurkat cells showed increase cytokine production, while treated cultures were not as productive. One possible explanation could be that cytokine production in this assay was an inverse correlation of the stimuli. As the stimuli (peptide treated cultures) was greater, the Jurkat culture cells decreased from over stimulation. Since Jurkat cells *per se* are already stimulated due to their tumorigenic properties, overstimulation will repress any activity. This reasoning is in accordance with ConA or peptide treated healthy or retrovirus infected cells (that are not hyperactivated

by oncogene activation, like in the case of Jurkat cells), showing higher cytokine production than the non-treated cell cultures.

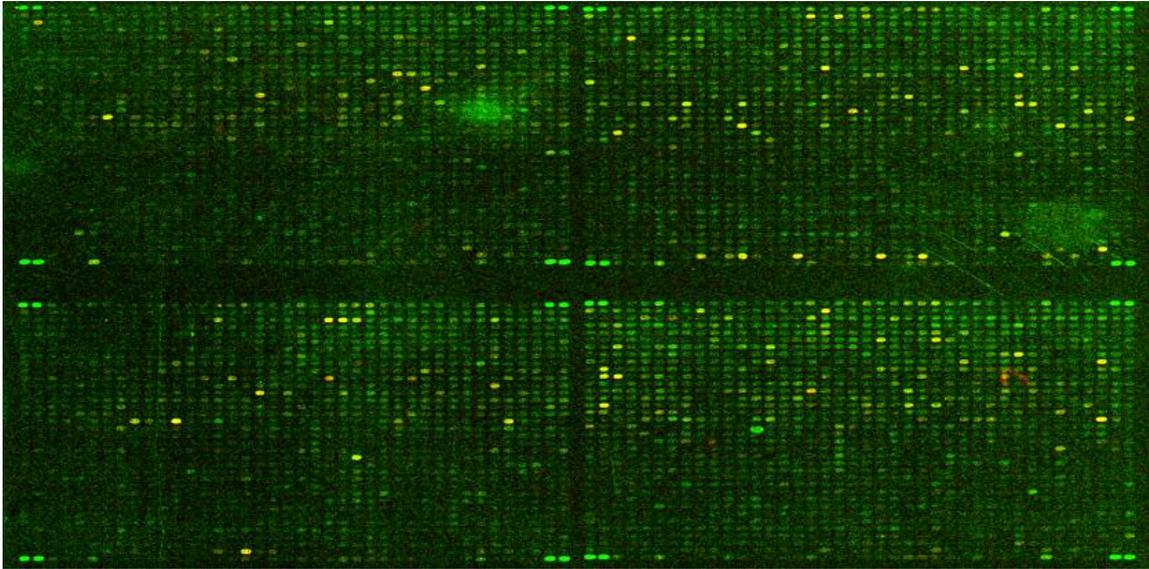


Figure 4.5: Expression patterns from Jurkat cell cDNA array. Jurkat cells were treated with the TCR V β 8.1 peptide for 48 h.

4.3 Discussion

Jurkat cells were considered ideal because *in vivo* studies have shown that the peptides influence T cell cytokine production, but it is not known if the peptide requires processing and presentation by antigen presenting cells (APC's). The use of a purified T cell line allows exclusion of the APC and evaluates the effect of the peptides on the T cell by itself. There is a clear inverse correlation between cell proliferation and TGF- β expression (a cell proliferation inhibitor). As the proliferation / mitogenesis assay shows (Fig.4.4), Jurkat cells treated with the TCR V β 8.1-peptide were more efficiently

proliferating. Nonetheless, TCR V β 8.1-peptide treated cells expressed lower cytokine intensity levels for all the cytokines determined. These results contradict the effect of TCR V β 8.1 peptide seen in mice where there is an increase in Th1 cytokine production. Healthy T cells do not usually produce IL-8. Miyamoto et al., 1997, has previously described that several cancer cell lines will produce high levels of IL-8 and that suppression of IL-8 production resulted not only in inhibition of cell growth, but also in an increase in the concentration of some tumor associated substances. In this experiment, the intensity levels for IL-8 production were exceptionally high in the Jurkat cells treated with TCR V β 8.1 peptide (86.02) while the other treated groups showed less intensity (Control: 6.12, ConA,19.32, and TCR V β 5.2: 4.41). Note that in this case, the intensity levels for IL-8 expression directly correlate with cell proliferation, Figure 4.4. Also, preliminary microarray analysis of Jurkat cells treated with the TCR V β 8.1 peptide has shown activation and inactivation of 53 genes. One of the genes that showed more than a 2 fold increase in expression was profilin 1 (AA521431). It has been demonstrated by others that profilin 1 expression is inversely correlated with the tumorigenic capacity of several cancers (breast cancer and breast epithelial cell lines), suggesting a general role for profilin 1 as a tumor suppressor protein (Janke et.al., 2000). This will suggest that even though the 8.1 peptide induces a higher proliferation rate, the tumorigenic capacity of the leukemia cells is decreased. These results correlate with studies done in our laboratory (Liang et al., 1988; Sepulveda et al., 2002a,b) where retroviral infected mice treated with the 8.1 peptide showed a longer life span but did not clear the infection.

Therefore, TCR V β 8.1 peptide might be contraindicated in conditions where tumor growth is exacerbated by IL-8 production. Future studies with TCR V β 5.2 could be performed to evaluate the improvement of the immune response in a host with cancer, without the risk of enhancing tumor growth. These results correlate with previously published data where TCR V β 5.2 and/or -8.1 given as therapy to mice with AIDS has proven effective in preventing the development of viral secondary infections but inefficient in diminishing the B cell lymphoma typically seen in the retrovirus infected mice (Liang et al., 1996; Marchalonis et al., 2001; Sepulveda et al., 2003).

It is not clear yet how the peptide binds to the T cell but the results show that they are capable of acting directly on the T lymphocyte and inducing biological changes.

CHAPTER 5

HEART GENE EXPRESSION DURING MURINE AIDS AND TCR V β 8.1 PEPTIDE TREATMENT

LP-BM5 retroviral induced immunodeficiency in C57/Bl6 mice is the murine model for human AIDS. Studies in our laboratory have shown that retroviral infection induces heart dysfunction as assessed by the Conductance Catheter System (CCS) (Liu et al., 2002; Yang et al., 1999). The inflammatory process in heart muscle may reflect host responses against the viral infection and ultimately result in congestive heart failure and dilated cardiomyopathy. On the other hand, host responses within the myocardium may secure the host against acute or protracted damage. TCR V β 8.1 peptide, a 16-mer peptide containing the entire CDR1 segment and part of the FR2 region of the human V β 8, has shown both an immunoregulating and immunostimulating effect in virally induced immunodeficient mice. To evaluate the modified gene expression of heart muscle tissue from immunodeficient mice with that of immunodeficient peptide treated animals, mRNA species were assessed in heart muscle using cDNA microarray technology. Expression levels were determined for more than 5,000 genes. Microarray analysis determined that there were more than 1,000 differentially expressed genes and 120 of these were found to differ by 2 fold. Analysis of these changes revealed a selective reprogramming of gene expression, particularly for 64-related genes, which were up or down regulated in the retrovirus infected mice treated with the TCR V β 8.1 peptide. Of importance are: Tubulin alpha 8, Actin-like 6, Myosin light chain, Muscle glycogen phosphorylase, selenoprotein P and Treonin reductase. The changes in the gene

expression suggest a mechanism for the loss of optimal heart function in retrovirally induced immunodeficient mice that may lead to heart failure and dilated cardiomyopathy and may explain the restoration of cardiac function after TCR V β 8.1 peptide therapy. These are the results of a pilot study.

5.1 Materials and Methods

5.1.1 Peptide

A set of overlapping 16-mer peptides that duplicate the covalent structure of the V β D β J β C β protein predicted from a human TCR-V β sequence has been produced. Here, we focus on the sequence C K P I S G H N S L F W Y R Q T that corresponds to the complete CDR1 and N-terminal five residues of Fr2 of the human V β 8.1 gene product. As a control peptide, we used a 16-mer corresponding to the CDR1 of the L chain MCG, because healthy mice did not produce autoantibodies (Aab) to this peptide. Its sequence is T G T S S D V G G Y N Y V S W Y. The peptide preparations were free of endotoxins. We have shown previously that normal polyclonal IgG pools contain natural Aab against peptide segments corresponding to CDR1, Fr3, and a constant region loop peptide of the TCR V β -chain.

5.1.2 cDNA Microarray Analysis

Total RNA was isolated from 1 whole heart using the Trizol method. Protocol was used to isolate total RNA. Healthy (Cy3) vs Retrovirus (Cy5) infected and Retrovirus infected (Cy5) vs Retrovirus infected + peptide treated animal hearts (Cy3). Fluorescent

first strand cDNA from each heart was made from 20 mg of total RNA using the Micromax Direct cDNA Microarray System. Each Cy5 labeled cDNA reaction was combined with one of the Cy3 labeled cDNAs using the Wiaquick PCR Purification Kit. After elution from the purification column, the probe was lyophilized to dryness, and resuspended in 15 ml hybridization buffer, denatured by boiling for 2.5 min, and added to a microarray. Co-hybridization of the mixture was done on purified PCR products of mouse cDNA inserts robotically spotted onto chemically activated glass microscope slides. Array was placed in a hybridization chamber at 62 C for 18 h. Slides were scanned for Cy3 and Cy5 fluorescence using an Axon GenePix 4000 microarray reader and quantitated using GenePix software. Gene expression results were analyzed using GeneSpring. Genes that showed at least a 1.5 –fold induction or a 0.5-fold reduction in expression were considered biologically important.

5.2 Results

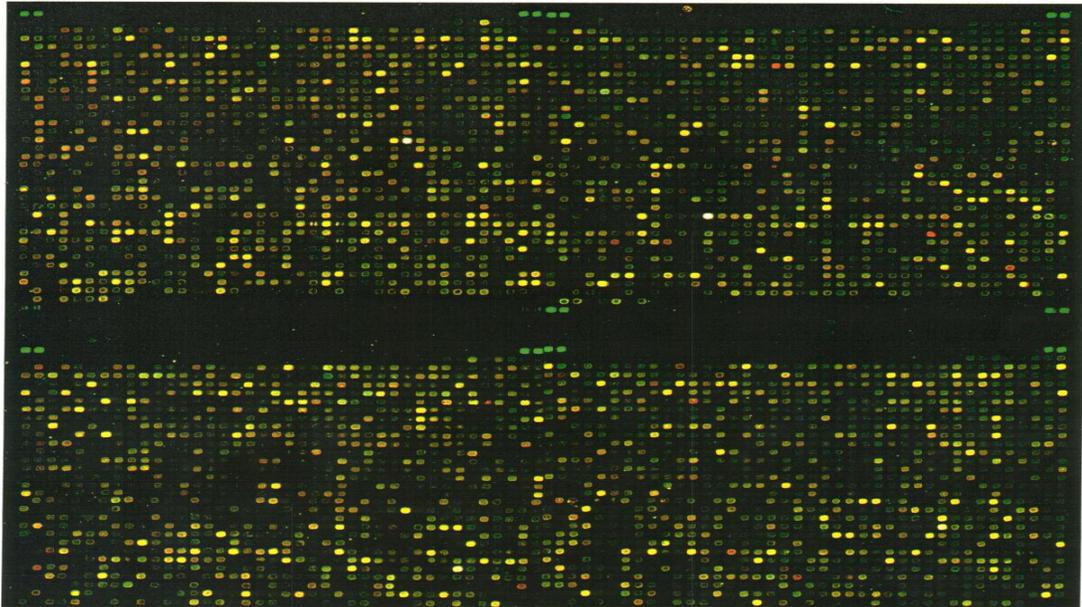


Figure 5.1: Expression patterns from mouse cDNA microarray. Heart muscle tissue from mouse infected with LP-BM5.

Table 5.1: Differential expression of genes at the mRNA level in hearts from retrovirus infected mice peptide and non-peptide treated. H = Healthy hearts; R = Hearts from retrovirus infected mouse; Rp = Hearts from retrovirus infected mouse treated with the peptide.

Gene	H vs R	R vs Rp	Gene exp
Coatomer protein complex sub	0.567	1.64305	UP
Solute carrier family 16	1.633	0.553	DOWN
Hemoglobin, beta adult m.ch.	2.591	0.508	DOWN
Peptidylglycine alpha-amid. M.	2.837	0.533	DOWN
Cadherin 1	1.555	0.654	DOWN
CD24a	1.222	0.251	DOWN
Ubiquitin-conjugating enzyme 8	2.965	0.561	DOWN
Hemoglobin Z, beta-like embry.	1.636	0.459	DOWN
Fetal liver zinc finger 1	0.894	1.522	UP
Dystrophia myotonica kinase	1.36	2.08	UP
Ribosomal protein S6 kinase	0.9194	1.708	UP
Interferon-inducible GTPase	4.52	1.766	DOWN
Cleft lip and palate associated t.	1.08	1.599	UP
Ring finger protein 13	1.0	1.51	UP
Immune associated nucleotide	0.91	1.98	UP
Procollagen, type IV, alpha 1	0.926	1.653	UP
Solute carrier family 25	1.15	2.04	UP
Glutamate oxalacetate trans.	1.38	1.799	UP
CUG triplet repeat, RNA-bin/pr	1.07	1.64	UP
Ubiquitin-like 3	1.01	1.55	UP
Protein kinase C and casein k.	1.02	2.04	UP
Lymphocyte antigen 116	1.41	2.44	UP
Bromodomain-containing 2	1.06	1.6	UP
Tuberous sclerosis 2	1.25	1.72	UP
NADH dehydrogenase flav.1	1.327	2.03	UP
Lipoprotein lipase	1.1	1.544	UP
Serine (or cys) protein	0.997	1.567	UP
Isocitrate dehydrogenase 3	1.13	1.86	UP
CD97 antigen	1.13	1.7	UP
Cristallin, alpha C	1.46	1.8	UP
Tubulin alpha 8	1.396	1.719	UP
Actin-like 6	1.16	2.01	UP
Histidine rich calcium b. p.	1.532	2.21	UP
Branched chain ketoacid dehy.	1.323	1.789	UP
Cadherin 2	1.248	1.522	UP

Table 5.1: Differential expression of genes at the mRNA level in hearts from retrovirus infected mice peptide and non-peptide treated-*continued*

ATP synthase, H ⁺ trans.	0.99	1.55	UP
Myosin light chain	1.03	1.63	UP
Muscle glycogen phosphorylase	1.5	2.29	UP
Glypican 1	1.0	1.61	UP
Cyclin D3	1.168	1.625	UP
Male enhanced antigen 1	1.46	2.417	UP
Troponin C, cardiac (Tncc)	0.975	1.537	UP
Enolasa 3, beta muscle	1.281	1.663	UP
Dystroglycan 1 (Dag 1)	1.20	1.747	UP

Table 5.2: Up regulated gene expression from heart muscle tissue of retrovirus infected mice treated with TCR V β 8.1 peptide.

Gene	Regulation
Tubulin alpha 8	UP
Actin-like 6	UP
Myosin light chain	UP
Muscle-glycogen-phosphorylase	UP
Selenoprotein P	UP
Threonine reductase	UP

5.3 Discussion

The differential gene expression phenomena presented in this study provides a framework for understanding the effect of retroviral induced immunodeficiency on heart integrity and functionality. Table 5.1 shows some of the approximately 1000 differentially expressed genes in the heart (at a 1.5-2.0 fold up or down expression) induced by the retroviral infection.

Clinical cardiomyopathy is seen in 1-4% of AIDS patients. In the United States, about 5 million people have heart failure and an additional 550,000 are diagnosed with it annually. It is one of the largest health problems in the developed world.

Cardiac disease has been reported in patients with AIDS based on clinical, echocardiographic and autopsy findings. In our laboratory, changes have been found in mice with AIDS using the CCS, measuring contractile functions, with end-systolic elastance and preload recruitable stroke work (both significantly decrease).

While abnormalities are often clinically silent, patients have developed congestive heart failure, cardiomyopathy, pericardial effusions and cardiac tamponade. Cardiac abnormalities may be caused by primary HIV infection of the myocardium, by superinfections or by the sequelae of drug therapy, substance abuse, renal impairment, or pulmonary disease. HIV-related cardiomyopathy is characterized by left ventricular dilatation and hypocontractility, which may result in heart failure and pulmonary congestion. It has been reported that depressed left ventricular fractional shortening (FS) and increased wall thickness (WT) are risk factors for mortality in children with HIV, independent of depressed CD4 cell count and neurological disease. FS has been

considered a long-term predictor of mortality while WT appears to be a short-term predictor.

Tubulin alpha 8 (TUBA8) is preferentially expressed in heart, skeletal muscle, and testis. Even though the precise biological function of TUBA8 is still unknown, it seems to have specific functional roles in cell cytoskeleton formation.

It is the interaction of myosin in the thick filaments with actin in the thin filaments that causes filament sliding and hence muscle contraction. This interaction is driven as a result of ATP hydrolysis by myosin. After myosin hydrolyses ATP to ADP and phosphate, it binds to actin and actin accelerates the release of phosphate. Phosphate release is closely coupled to the power stroke, in which myosin heads pull on the actin filament to produce force or movement. In the adult human heart, two isoforms of myosin are expressed. The predominant isoform is β -myosin heavy chain, but a small amount of α -myosin heavy chain is also present. These 2 isoforms can be distinguished on the basis of the speed with which they hydrolyse ATP; the α isoform has 2-3 times the actin-activated ATPase activity of the β -isoform. Myosin isoform expression is plastic and can change throughout life as a result of disease or merely as a consequence of aging.

Glycogen is a high molecular weight polysaccharide that serves as a repository of glucose units for utilization in times of metabolic need. Cardiac glycogen is present at high levels during early to midgestation before falling to low levels at the time of birth. The fact that glycogen is present and that its level changes during the embryonic development of several tissues suggest that it may have a specific role- congenital heart disease is one of the most common birth defects in newborns. Reports of the incidence of

moderate to severe defects range from 6 to 19 per 1,000 live births. Studies have shown that glycogen plays a critical role as an energy source during cardiogenesis and that impaired glycogen synthesis may contribute to congenital heart disease.

HIV associated cardiomyopathy may be related either to a direct action of HIV on the myocardial tissue or to an autoimmune process induced by HIV in association with other cardiotropic viruses. An alternative could be that immune dysregulation caused by the retroviral infection induces myocardial dysfunction. Infants born to mothers infected with HIV sustain cardiac damage regardless of whether they contract the viral infection. Alterations in contractile protein expression (in both actin and myosin) have been seen to be induced by cytokine changes (Table 5.2). TCR V β 8.1 peptide therapy, a well known immunoregulator is a promising novel approach for AIDS related cardiopathology.

CHAPTER 6

CONCLUSIONS

The studies reported here follow from findings that normal humans and mice have low levels of autoantibodies to peptide defined TCR V β epitopes, and that levels and isotypes of these can vary with infection, particularly retroviral infection, as well as aging and autoimmune diseases.

- Administration of the TCR V β 8.1 peptide had a restorative effect on T-cell function, particularly that of Th1 cells, maintaining nearly normal functioning of general parameters of immune responses.

- TCR V β 8.1 peptide treatment induces B and T cell proliferation.

- TCR V β 8.1 peptide treatment of a purified cell line (Jurkat cell) induces changes in cytokine expression.

- Administration of the peptide restored a balance of Th1 and Th2 cytokine activities by increasing the former and decreasing the latter relative to one another.

- Administration of the TCR V β 8.1 peptide tends to generate Th1-type T cells, suppressing the deleterious effect of the Th2 cells that are proposed to cause heart pathology due to CVB3 infection.

- TCR V β 8.1 peptide induces proliferation and production of IL-2 in splenocytes from naïve and retrovirus infected mice.
- TCR V β 8.1 peptide shows the apparent capacity to synergize with low levels of the T- and B-cell mitogens Concavalin A and LPS to, respectively, initiate cell proliferation.
- Coxsackievirus B3/59 induces severe myocarditis in retrovirus infected immunodeficient mice.
- An avirulent strain of coxsackievirus, CVB3/0, isolated from retrovirus infected immunodeficient mice, develops cardiovirulence after one passage.
- TCR V β 8.1 peptide protects against CVB3 induced cardiopathology in retrovirus infected mice.
- Prophylactic treatment with TCR V β 8.1 peptide of retrovirally infected mice increases survival.
- Prophylaxis with TCR V β 8.1 peptide of retrovirus infected mice co-infected with CVB3 increases survival.

- Prophylaxis with TCR V β 8.1 peptide of retrovirus infected mice co-infected with the cardiovirulent strain CVB3 decreases severity of heart pathology.
- Prophylactic treatment with TCR V β 8.1 peptide of retrovirus infected mice promotes an increase in gene expression of molecules that participate in heart function.

6.1 Future Studies

Type 1 diabetes (T1D) is caused by a progressive destruction of insulin producing beta (β) cells in the pancreas leading to insulin deficiency and hyperglycemia. The pathogenesis is mediated by autoimmune mechanisms reflected by lymphocyte infiltration in the islets of Langerhans and autoantibodies against β -cell autoantigens. Survival analyses between the mid 1940's and mid 1960's indicates that 20% of type 1 diabetes cohorts die within 25 years after diagnosis and fewer than 40% survive 50 years of the disease. The incidence of T1D over the past 50 years has been increasing 3-5% annually. Since the rise in the incidence is too rapid from one generation to the next to be explained by genetic susceptibility alone, the role played by changing environmental factors has become important in understanding the mechanism of T1D. Diet, toxins and viruses have been agents identified with triggering islet autoimmunity culminating in T1D. Coxsackievirus B4 (CVB4) has been identified as one such causative agent of T1D in humans. The period between the development of insulinitis and the onset of T1D

correlates with an imbalance of T regulatory (Tr) cells. Spontaneous onset of T1D in non-obese diabetic (NOD) mice results from the loss of immunoregulation with a dominance of pathogenic Th1 cells over protective Th2 cells. Peptide based therapies using sequences derived from T-cell receptors (TCR) have been shown to prevent or delay the onset of autoimmune diseases. As stated previously, we have shown that administration of TCR V β 8.1 peptide has a restorative effect on mouse T-cell function after viral induced immunodeficiency and immunosenescence. Three specific aims can be pursued: (1) Evaluate the immunoprotective effect of the TCR peptide against T1D development using a healthy susceptible CD1 mouse infected with CVB4; (2) Characterize the pathogenesis and immunological status in retrovirally induced immunodeficient mice when co-infected with CVB4 and treated with TCR peptide and, (3) Examine the role of TCR peptide in spontaneous T1D using NOD mice to characterize the effect of the peptide on the humoral and cellular immune response. These studies will provide critical information on the immunoregulatory properties of the peptide as an alternative or joint therapeutic strategy for T1D.

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