Prevention of Retrovirus-Induced Aberrant Cytokine Secretion, Excessive Lipid Peroxidation, and Tissue Vitamin E Deficiency by T Cell Receptor Peptide Treatments in C57BL/6 Mice (44074)

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Abstract. **To test whether T cell receptor (TCR) peptide treatment can prevent immune dysfunction, excessive lipid peroxidation, and malnutrition caused by retrovirus infection, female C57BL/6 mice were infected with LP-BM5 retrovirus. Infection with retrovirus inhibited lymphocyte proliferation, cytokine release T helper 1 cells, stimulated cytokine secretion by T helper 2 cells, induced abnormal hepatic and cardiac lipid profiles, and produced excessive tissue lipid peroxidation with hepatic and cardiac vitamin E deficiency. Two weeks after infection, TCR peptides Vβ5.2, Vβ8.1, Vβ8.1 + Vβ5.2, Vβ8.1(N), and Vβ8.1© were injected to the mice at dose of 200 μg/mouse. Vβ8.1 and Vβ5.2 treatments largely maintained lymphocyte proliferation and IL-2 and IFN-γ release, and prevented excessive IL-6, IL-10, and TNF-α secretion. Concomitantly, these treatments normalized hepatic and cardiac lipid profiles, reduced tissue lipid peroxidation, and thereby significantly maintained vitamin E in the liver and heart. Vβ8.1 segments treatment did not prevent the immune dysfunction, abnormal lipid profile and lipid peroxidation, and vitamin E deficiency caused by the retrovirus infection. In conclusion, injection of intact TCR peptides during murine retrovirus infection largely prevented immune dysfunction by blocking the excessive stimulation of a T cell subset caused by retroviral superantigens. It also ameliorated malnutrition status by normalizing lipid profile, lipid peroxidation, and vitamin E deficiency. T cell immune dysfunction and its prevention by TCR peptide treatment is important in the therapy of vitamin E deficiency induced by retrovirus infection.**
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Murine acquired immune deficiency syndrome (AIDS) is induced by infection with the LP-BM5 murine leukemia retrovirus mixture. It shares many similarities to the pathogenesis of human AIDS, even though human immunodeficiency virus (HIV) and LP-BM5 murine leukemia virus (MuLV) represent different types of retrovirus (1). Murine AIDS is characterized by lymphadenopathy, splenomegaly, hypergammaglobulinemia, deficient B cell response to T-independent antigens *in vitro*, reduced T cell functions, loss of disease resistance, impaired cytokine production, and tissue vitamin E deficiency (1).

Anorexia, weight loss, and complications of recurrent infections in AIDS patients frequently progress to multiple nutrient deficiencies and protein energy malnutrition (2), which could accelerate immunosuppression. Superoxide radicals including hydrogen peroxides, hydroxyl radicals, and lipid peroxides are produced at high levels when immune defenses are breached with increased exposure to bacterial mitogens and endotoxins. These highly reactive oxygen-containing molecules may facilitate disease progression from HIV infection

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to AIDS (3) by their reaction with antioxidant vitamins, exacerbating nutritional deficiency as well as directly inducing immunosuppression.

Vitamin E (4) may be an important immune modulator as tissue levels of vitamin E are reduced by immune dysfunction during murine AIDS (5–7). In uninfected mice, vitamin E supplementation (15 times of basal content 500 IU/g) increased the $CD4^+/CD8^+$ ratio and total lymphocytes, and stimulated activity of cytotoxic cells, natural killer (NK) cell activity, T- and B-cell mitogen responsiveness, and phagocytosis by macrophages (4, 8). Vitamin E deficiency could accentuate the immunosuppression of the retrovirus infection as supplementation of vitamin E partially restored immune function in retrovirus-infected mice (5–7). The loss of vitamin E may be due to murine retrovirus-induced immune dysfunction (5), resulting in increased production of free radicals and lipid peroxides, which are immunosuppressive and could accelerate development of murine AIDS.

Immunological methods preventing retrovirus-induced immune dysfunction have been studied to assert their inhibiting effects on the excessive lipid peroxidation and loss of vitamin E during infection. T-cell receptor (TCR) peptide treatment largely prevented the loss of immune function during retroviral infection by blocking the interaction of retroviral superantigens with a subset of T cells (9). This treatment stopped the T-cell subset from being stimulated to become activated T-helper 2 (Th2) cells whose excessive cytokine production suppresses T-helper 1 (Th1) cells and cellular immunity. The response of autoantibodies to the TCR peptides, elevated to regulate the activated T cells subsets, was stimulated by the murine retrovirus (10). Infected mice made high levels of antibodies against two human TCR peptides to suppress T cells bearing homologous murine Vβ peptides. Thus these two human peptides were identified as important factors in the mouse's attempts to regulate and suppress specific T-cell subsets, excessively stimulated by retroviral superantigen (10). By stopping stimulation of this subset by retroviral superantigens, TCR peptide treatment prevented T-helper 0 (Th0) cells' conversion to Th2 cells and excessive production of Th2 cytokines (9). Hyperproduction of Th2 cytokines suppresses neighboring Th0 and Th1 cells and induces immune suppression. While several TCR peptides are good immunogens, the peptides used to treat murine AIDS were not, even when used with adjuvants (9). Thus, additional antibodies did not develop against this Vβ peptides after their injection into the infected mice even in the presence of adjuvant. Therefore, the TCR peptide prevents excessive stimulation of the Th2 cells by retroviral superantigens by an unknown mechanism and their production of large amounts of IL-4, -6, and -10 (9). Th2 cytokines suppress Th1 cells, causing anergy of cell mediated immunity, allowing the retrovirus to continue to reproduce and stimulate oxidative radical secretion by macrophages (11).

The current study tested whether treatment with different TCR Vβ CDR1 peptides or fragments of the peptides would balance cytokine production, reduce oxidative damage, lipid peroxidation, and thus prevent the loss of tissue vitamin E during the LP-BM5 retrovirus infection.

Methods and Materials

Animals and Murine AIDS. Female C57BL/6 mice, 4 weeks old, were obtained from the Charles River Laboratories Inc. (Wilmington, DE) and housed in transparent plastic cages with stainless steel wire lids (four mice per cage) in the animal facility of the Arizona Health Science Center. Animals were cared for as required by the University of Arizona Committee on Animal Research. The housing facility was maintained at 20°–22°C and 60%–80% relative humidity, with a 12:12-hr light:dark cycle. Water and semipurified diet (4% mouse diet, #7001; Teklad, Madison, WI) were freely available. After 2 weeks of housing, the mice in the dose and adjuvant studies were randomly assigned to the following treatments with eight mice per group: uninfected, normal mice injected with saline (pyrogen free); LP-BM5–infected, normal mice injected with saline (pyrogen free); LP-BM5–infected mice injected with 200 μg TCR internal Vβ 5.1 control peptide; LP-BM5–infected mice injected with 200 μg TCRVβ8.1 pep β3 CDR1 peptide; LP-BM5–infected mice injected with 200 μg TCRVβ5.2 CDR1 peptide; LP-BM5–infected mice injected with 200 μg TCRVβ8.1 pep β3 and 200 μg Vβ5.2 CDR1 peptide; LP-BM5–infected mice injected with 200 μg TCR pep β3(N) CDR1 peptide segment; LP-BM5–infected mice injected with 200 μg TCR pep β3© CDR1 peptide segment.

LP-BM5 retrovirus was administered intraperitoneal to mice in 0.1 ml minimum essential medium (MEM) medium with an ecotropic titer (XC) of 4.5 $\log_{10}$ plaque forming units $\times 10^{-3}$/l, which induces disease with a time course comparable to that previously published (1). Administration of peptides (dissolved in saline) and adjuvants was performed 2 weeks after LP-BM5 infection. Uninfected mice were injected with MEM used for LP-BM5 virus growth as controls. Infection of adult female C57BL/6 mice with LP-BM5 MuLV leads to the rapid induction of clinical symptoms with virtually no latent phase (1).

The infection and treatment period was 14 weeks for all groups. Mice were sacrificed while under ether anesthesia. Spleens and lymph nodes were then dissected, removed, and kept at 4°C. Livers and hearts for nutritional analysis were collected and stored at −70°C until assayed.

Peptides. A set of overlapping 16-mer peptides that duplicate covalent structure of the TCR β product (12, 13) predicted from the human JURKAT sequence (14) has been produced (Table I). An effective immunomodulatory peptide has the sequence C K P I S G H N S L F W Y R Q T, which corresponds to the complete CDR1 and N-terminal five residues of Fr2 (12, 13) of the human Vβ8.1 gene

Table I. T-Cell Receptor Vβ Synthetic Peptides Used in the Study

Description	Sequence	Designation
CDR1 of Vβ8.1	C K P I S G H N S L F W Y R Q T	Vβ8.1
N terminus of Vβ8.1	C K P I S G H N S L F	Vβ8.1(N)
C terminus of Vβ8.1	S G H N S L F W Y R Q T	Vβ8.1(C)
CDR1 of Vβ5.2	C S P K S G H D T V S W Y Q Q A	Vβ5.2
Internal Vβ5.1	S P R S G D L S V Y	INT 5.1

product (14). Two segments of Vβ8.1 were used in this study. They were the N-terminal of the Vβ8.1 and C-terminal of the Vβ8.1 (15). A peptide corresponding to the sequence of the 16 mer of the Vβ5.2 peptide gene product, C S P K S G H D T V S W Y Q Q A, was synthesized as a homolog often recognized by autoantibodies. Normal polyclonal IgG pools contain natural AAbs against peptide segments correspond to CDR1, Fr3 and to a constant region "loop" peptide (12). Untreated mice also have natural IgG antibodies directed against the same peptide segments; in particular, there is strong reactivity to the human CDR1 test peptides (15). A computer comparison of human and murine Vβ sequences (Marchalonis, unpublished analysis) using the progressive alignment algorithm of Feng and Doolittle (16) showed that certain human and murine Vβ sequences could be grouped into families (e.g., human Vβ6 and Vβ8 correspond to murine Vβ11, and human and murine Vβ5 are in the same clusters).

Determination of Conjugated Dienes and Lipid Fluorescence. Approximately 0.5 g of tissue was homogenized in 10 ml of Folch solution (2:1, v/v chloroform: methanol). After protein separation, a 0.1-ml fraction was dried in a steady flow of nitrogen gas at 55°C and used to determine conjugated dienes and lipid fluorescence as previously described (17). The residue was redissolved in methylene chloride and washed twice with water. To the methylene chloride solution was added 0.5 ml methanol to clarify the emulsion. Conjugated diene fatty acids were determined by obtaining absorbency of the solution at 237 nm in a Beckman DU-7 recording spectrophotometer (Fullerton, CA) using an appropriate blank. Lipid fluorescence of the homogenate was measured in a Hitachi F-2000 fluorescence spectrophotometer (Hitachi Ltd, Tokyo) with a setting of fluorescence maximum at 470 nm and an activation wavelength of 395 nm. Details of the methods used have been previously described (18).

Determination of Phospholipid. The phospholipid content of the livers and hearts was determined by the method of Raheja *et al.* (19). This method does not required the predigestion of the phospholipid. Dipalmitoyl phosphatidylcholine was used as a standard.

Determination of Vitamin E. Vitamin E levels in liver and heart tissues were measured by high-pressure liquid chromatography as described previously (20). Briefly, approximately 0.1 g of tissue was homogenized in 1 ml of water. Butylated hydroxytoluene was added to prevent oxidation of α-tocopherol. Pentane, ethanol, and sodium dodecyl sulfate were used to extract α-tocopherol from the homogenate. Extracts was evaporated under steady flow of nitrogen gas at 20°C and then redissolved in 0.5 ml methanol injection onto a C18 column (3.9 × 150 mm NovaPak; Millipore, Bedford, MA). A mobile phase composed of methanol and sodium acetate in the ratio of 98:2 (by volume) at a flow rate of 1.5 ml/min was used. α-Tocopherol, eluted at 6.5 min, was monitored by a fluorescence detector (Millipore, Bedford, MA) at 290 nm excitation and 320 nm emission wavelength.

Standard Cytokines and Their Antibodies. Rat anti-murine IFN-γ, IL-2, IL-6, and IL-10 purified antibodies; rat anti-murine IFN-γ, IL-2, IL-6, and IL-10 biotinylated antibodies; and recombinant murine IFN-γ, IL-2, IL-6, and IL-10 were obtained from Pharmingen (San Diego, CA).

ELISA for Cytokines. The production of IFN-γ, IL-2, IL-6, and IL-10 from mitogens-stimulated splenocytes was determined as described previously (21). Briefly, spleens were gently teased with forceps in culture medium (CM, RPMI 1640 containing 10% fetal calf serum, 2 m*M* glutamine, 1×10^5 units/l penicillin and streptomycin), producing a suspension of spleen cells. Red blood cells were lysed by the addition of a lysis buffer (0.16 *M* ammonia chloride Tris buffer, pH 7.2) at 37°C for 3 min. Then the cells were washed twice with CM. Cell concentrations were counted and adjusted to 1×10^{10} cells/l. Splenocyte viability was more than 95% as determined by trypan blue exclusion. Cultured in triplicate on 96-well flat-bottom culture plates (Falcon 3072, Lincoln Park, NJ) with CM were 0.1 ml/well of splenocytes (1×10^{10} cell/l). The splenocytes were then stimulated with concanavalin A (Con A, 1×10^{-2} g/l, 0.1 ml/well, Sigma Chemical Co., St. Louis, MO) to determine their production of IL-2 and IL-10 after 24 hr of incubation, IFN-γ after 72 hr of incubation in a 37°C, 5% CO_2 incubator. Splenocytes were also incubated for 24 hr after the addition of lipopolysaccharide (LPS, 1×10^{-2} g/l; Gibco, Grand Island, NY) to induce IL-6 and TNF-α production. After incubation, the plates were centrifuged for 10 min at 800 *g*. Supernatants were collected and stored at −70°C until analysis. The cytokines were determined by sandwich enzyme-linked immunosorbent assay (ELISA) as described previously (21).

Statistics. All parameters were compared using a one-way analysis of variance (ANOVA), followed by a *t*

test (two-sample assuming unequal variances) for comparison between any two groups. $P < 0.05$ was considered to be significantly different between two groups.

Results

Body Weight. Body weights were not affected by various TCR Vβ CDR1 peptide treatments post retrovirus infection (data not shown). There was no significant change in food consumption due to infection or peptide treatments (data not shown). The spleen and lymph node weights (14 weeks postinfection) were significantly ($P < 0.05$) elevated in the infected mice (data not shown), which indicated that infection had progressed to murine AIDS (1). However, none of the peptides significantly prevented the increase in spleen weight.

Hepatic and Cardiac Vitamin E. The liver and heart are the major organs which have been studied for tissue vitamin E deficiency in murine AIDS (5–7). Hepatic and cardiac vitamin E was significantly ($P < 0.05$) reduced by retrovirus infection (Fig. 1 and Table II). TCR Vβ8.1 and Vβ5.2 peptides treatment significantly ($P < 0.05$) retarded the loss of tissue vitamin E during infection (Fig. 1 and Table II). Infected mice administered TCR Vβ8.1, Vβ5.2, and Vβ8.1 + Vβ5.2 peptides had significantly ($P < 0.05$) higher hepatic and cardiac vitamin E levels than infected mice without TCR peptide treatment. Retrovirus-infected mice that received TCR Vβ8.1(N) and Vβ8.1© peptide segments treatment had significantly lower ($P < 0.05$) hepatic and cardiac vitamin E levels than uninfected mice as well as infected mice injected with TCR Vβ8.1 and Vβ5.2 peptides, alone and combined. Mice treated with the combination of Vβ8.1 + Vβ5.2 peptides treatment maintained hepatic and cardiac vitamin E levels near those of uninfected mice.

Hepatic and Cardiac Lipid Peroxidation. Diene conjugates and lipid fluorescence are the major products of lipid peroxidation. Significantly ($P < 0.05$) higher hepatic and cardiac diene conjugates and lipid fluorescence levels were induced by retrovirus infection (Fig. 2, A and B; Table II). Treatment with TCR Vβ8.1 and Vβ5.2 significantly ($P > 0.05$) retarded the excessive production of diene conjugates and lipid fluorenscence in the liver and heart during infection (Fig. 2 A and B; Table II). Infected mice administered TCR Vβ8.1, Vβ5.2, and Vβ8.1 + Vβ5.2 peptides had significant ($P < 0.05$) lower hepatic and cardiac diene conjugates and lipid fluorescence levels than that of infected mice without TCR peptide treatment. Retrovirus-infected mice that received TCR Vβ8.1(N) and Vβ8.1© peptide segments treatment had significantly higher ($P < 0.05$) hepatic and cardiac diene conjugates and lipid fluorescence levels than uninfected mice as well as infected mice treated with TCR Vβ8.1 and Vβ5.2. The mice given the combination of Vβ8.1 + Vβ5.2 peptides maintained hepatic and cardiac diene conjugates and lipid fluorescence levels near those of uninfected mice.

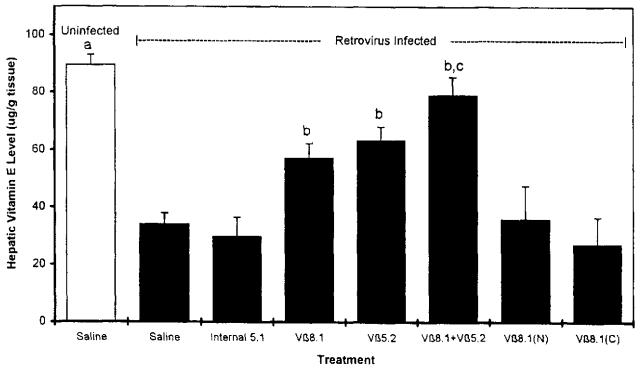

Figure 1. Effect of different T cell receptor peptide treatments on hepatic vitamin E level. Values are mean ± SD; $n = 8$. Letters indicate significant differences at $P < 0.05$: [a]compared with all of the retrovirus-infected groups; [b]compared with the retrovirus-infected groups injected with saline, internal 5.1 peptide, Vβ8.1(N), and Vβ8.1(C) peptide; [c]compared with the retrovirus-infected groups injected with Vβ8.1 and Vβ5.2 peptide alone.

Hepatic Lipid Profiles—Phospholipid, Triacyclglycerol, and Cholesterol Levels. Lipid profiles significantly affect fat-soluble vitamin E levels in tissue. Significantly ($P < 0.05$) higher hepatic phospholipid, triacyclglycerol, and cholesterol levels were caused by retrovirus infection (Fig. 3, A–C). TCR Vβ8.1 and Vβ5.2 peptides treatment significantly ($P < 0.05$) retarded the excessive accumulation or synthesis of phospholipid, triacyclglycerol, and cholesterol in the liver during infection (Fig. 3, A–C). Infected mice administered TCR Vβ8.1, Vβ5.2, and Vβ8.1 + Vβ5.2 peptides had significant ($P < 0.05$) lower hepatic phospholipid, triacyclglycerol, and cholesterol levels than that of infected mice without TCR peptide treatment. Retrovirus-infected mice that received TCR Vβ8.1(N) and Vβ8.1© peptide segments had significantly higher ($P < 0.05$) hepatic phospholipid, triacyclglycerol, and cholesterol levels than uninfected mice as well as infected mice injected with TCR Vβ8.1 and Vβ5.2. The mice treated with the combination of Vβ8.1 + Vβ5.2 peptides maintained hepatic phospholipid, triacyclglycerol, and cholesterol levels near those of uninfected mice.

Immune Function Analysis. IL-2 and IFN-γ are secreted by Th1 lymphocytes and module cell-mediated immunity. IL-6, IL-10, and TNF-α are produced by Th2 lymphocytes and regulate humoral responses while suppressing Th1 cells (22). Significantly ($P < 0.05$) lower Th1 cytokines levels (Fig. 4A; data not shown for IFN-γ) and excessive Th2 cytokines levels (Fig. 4B; data not shown for IL-6 and IL-10) were induced by retrovirus infection. TCR Vβ8.1 and Vβ5.2 peptides treatment significantly ($P < 0.05$) normalized cytokine production by Th1 cells and retarded the excessive production of cytokine by Th2 cells during infection. Infected mice administered TCR Vβ8.1, Vβ5.2, and Vβ8.1 + Vβ5.2 peptides had significant ($P < 0.05$) higher Th1 cytokines production and lower Th2 cytokines production than did infected mice without TCR peptide treatment. Retrovirus-infected mice that received TCR Vβ8.1(N) and Vβ8.1© peptide segments treatment had significantly ($P < 0.05$) lower Th1 cytokines production and higher Th1 cytokines production than uninfected mice as well as infected

Table II. Cardiac Vitamin E, Diene Conjugates, and Lipid Fluorescence Levels

Treatment	Vitamin E (μg/g tissue)	Diene conjugates (absorbance units/mg phospholipid)	Lipid fluorescence (Fluorescence units/mg phospholipid)
Uninfected + saline	69.2 ± 3.1[a]	1.9 ± 0.6[a]	12.7 ± 2.8[a]
Infected + saline	12.9 ± 3.5	6.7 ± 0.9	36.8 ± 2.3
Infected + internal 5.1	15.7 ± 5.7	6.1 ± 1.5	39.9 ± 5.9
Infected + Vβ8.1	38.3 ± 6.1[b]	3.5 ± 1.1[b]	29.1 ± 4.3[b]
Infected + Vβ5.2	45.1 ± 5.8[b]	3.2 ± 1.3[b]	26.4 ± 4.1[b]
Infected + Vβ8.1 + Vβ5.2	62.8 ± 7.3[b,c]	2.1 ± 0.6[b,c]	18.3 ± 5.4[b,c]
Infected + Vβ8.1 (N)	21.5 ± 12.8	5.4 ± 1.7	35.5 ± 9.7
Infected + Vβ8.1 (C)	16.3 ± 10.6	7.5 ± 1.9	31.0 ± 10.2

Note. Every sample from each mouse was measured in triplicate. Values are mean ± SD; $n = 8$. Letters indicate significant differences at $P < 0.05$: [a]compared with all of the retrovirus-infected groups; [b]compared with the retrovirus-infected groups with TCR Vβ8.1, Vβ5.2, and Vβ8.1 + Vβ5.2 peptide treatment; [c]compared with the retrovirus-infected groups with single TCR Vβ8.1 and TCR Vβ5.2 peptide treatment.

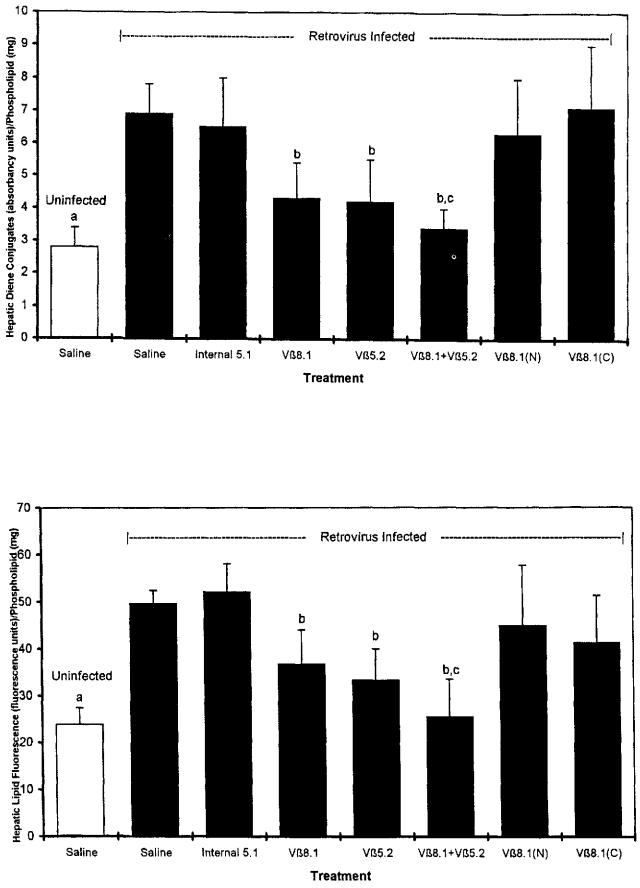

Figure 2. Effect of different T cell receptor peptide treatments on hepatic diene conjugates level (A) and on hepatic lipid fluorescence level (B). Every sample from each mouse was determined in triplicate. Values are mean ± SD; $n = 8$. Letters indicate significant differences at $P < 0.05$: [a]compared with all of the retrovirus-infected groups; [b]compared with the retrovirus infected groups injected with saline, internal 5.1 peptide, Vβ8.1(N), and Vβ8.1(C) peptide; [c]compared with the retrovirus infected groups injected with Vβ8.1 and Vβ5.2 peptide alone.

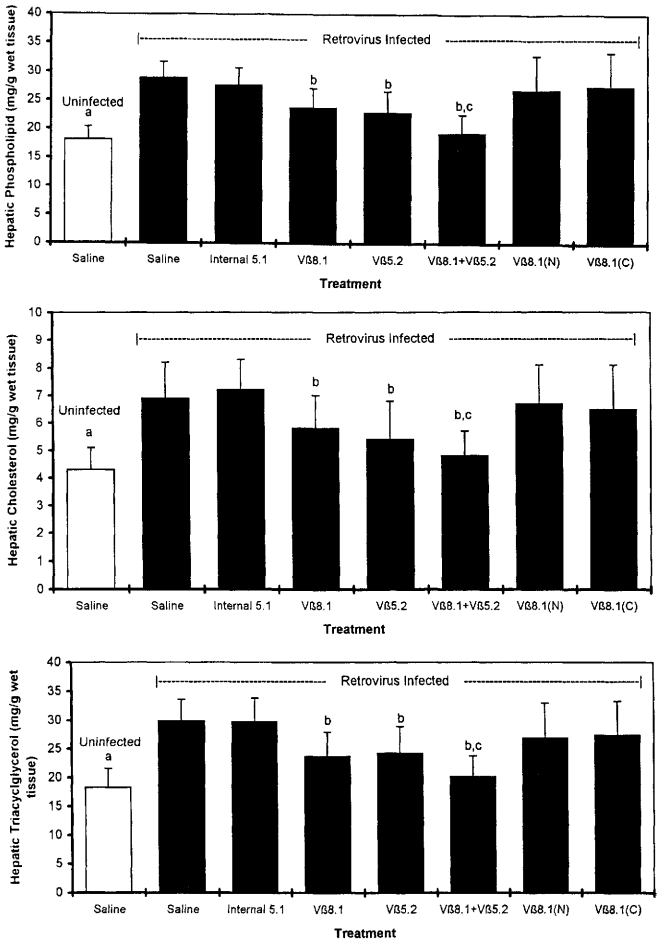

Figure 3. Effect of different T cell receptor peptide treatments on hepatic phospholipid level (A), triacylglycerol level (B), and cholesterol level (C). Every sample from each mouse was measured in triplicate. Values are mean ± SD; $n = 8$. Letters indicate significant differences at $P < 0.05$: [a]compared with all of the retrovirus-infected groups; [b]compared with the retrovirus-infected groups injected with saline, internal 5.1 peptide, Vβ8.1(N), and Vβ8.1(C) peptide; [c]compared with the retrovirus-infected groups injected with Vβ8.1 and Vβ5.2 peptide alone.

mice injected with TCR Vβ8.1 or Vβ5.2. The mice given the combination of Vβ8.1 + Vβ5.2 peptides treatment maintained Th1 and Th2 cytokines production near those of uninfected mice.

Discussion

Our studies help clarify the relationship between loss of vitamin E, increased lipid peroxidation, and immune dysfunction caused by murine retrovirus infection. TCR pep-

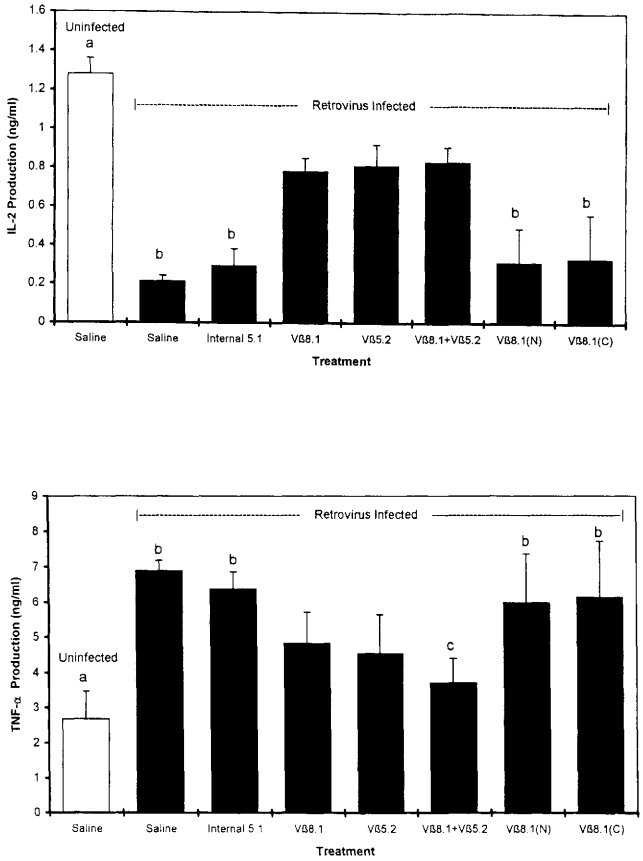

Figure 4. Effect of different T cell receptor peptides treatment on interleukin-2 (A) and tumor necrosis factor–α (B) production by splenocytes from *in vitro*. Every sample from each mouse was measured in triplicate. Values are mean ± SD; $n = 8$. Letters indicate significant differences at $P < 0.05$: [a]compared with all of the retrovirus-infected groups; [b]compared with retrovirus-infected groups with TCR Vβ8.1, Vβ5.2, and Vβ8.1 + Vβ5.2 peptide treatment; [c]compared with the retrovirus infected groups with single TCR Vβ8.1 and TCR Vβ5.2 peptide treatment.

tide treatments simultaneously prevented immune dysfunction and the loss of tissue vitamin E (11). The current studies demonstrated that treatment with TCR Vβ CDR1 peptides significantly decreased the evidence of oxidative stress associated with murine retrovirus infection. TCR peptide treatment significantly reduced immune dysfunction, oxidative damage in tissue, and loss of tissue vitamin E. Although the interaction between immune function and nutritional status still needs further elucidation, maintenance of immune function and tissue vitamin E levels occurred concomitantly with prevention of the stimulation of a T cell clone induced by murine retrovirus superantigens (9). However, fragments representing 10 amino acids of a 16–amino acid sequence from either the N terminus or C terminus of the active peptide Vβ8.1 did not retain activity in preventing immune dysfunction or loss of vitamin E. Thus, fragments of the CDR1 segment or the reading frame segment only did not maintain activity. Both the CDR1 portion and the reading frame component of the peptide are necessary for activity, or the peptide must be longer than 10 amino acid to be functional.

Most antigens are recognized through their interaction with the variable V portions of the TCR α- and β-chains. T cells also recognize superantigens, which interact with the Vβ region alone, independently from other variable TCR components. $CD4^+$ T-cell expansion or depletion requires the stimulation of T-cell subgroups by chronic or superretroviral antigens. Over time, this results in excessive activation of $CD4^+$ T cells bearing superantigen selected Vβ's followed by a general anergy. Autoantibodies against Vβ peptides, found in high levels in infected mice (10), defined the TCR epitopes used to select the two peptides for our studies. Similarly autoantibodies against some TCR Vβ peptides were high in AIDS patients. Our previous data (9) showed that TCR Vβ8.1 peptide treatment prevented immune dysfunction, indicating that this peptide could be considered an immunoregulatory element in the complex network of interactions between components of the immune response.

Our data suggest an association among immune dysfunction, lipid peroxidation, and tissue vitamin E. Immune dysfunction during murine retrovirus infection decreases the host resistance to opportunistic pathogens, facilitating infection and pathogen reproduction. The resulting increased antigen levels stimulate phagocytes to release more free radicals and increase lipid peroxidation. Prevention of immune dysregulation by the various TCR peptides simultaneously eliminates excessive lipid peroxidation and reduces the loss of tissue vitamin E. Similarly maintenance of immune function in retrovirus-infected mice occurred when early treatment with large doses of the TCR Vβ8.1 peptide was conducted, which prevented development of high level of lipid peroxidation while retaining tissue vitamin E (11).

HIV^+ patients have reduced serum vitamin E levels at various stages of the disease (23). Most patients who had AIDS (50%), who had AIDS-related complex (58%), and/or who were HIV-infected (38%) had a vitamin E intake of less than 50% of the Recommended Daily Allowance (24). However, reduced tissue levels of vitamin E were not due to the lower intake during murine retrovirus infection as deficiencies of vitamin E occurred in the liver, spleen, and thymus (6, 7), even though the mice consumed the recommended amount of vitamin E. Vitamin E–deficient rats have depressed antibody-dependent cell cytotoxicity (25), lymphocytes blastogenesis in response to mitogens (26), and natural killer cell–mediated cytotoxicity (27). Thus, immunological defects related to retrovirus infections could be exacerbated by retrovirus-induced vitamin E deficiency. Supplementation with high levels of vitamin E during murine retrovirus infection restored tissue vitamin E levels, while it partially normalized immune dysfunction (5).

Vitamin E inhibits oxidation of cellular components by free radicals and singlet oxygen, and is the most effective antioxidant at higher partial pressures (28). Vitamin E is an immune enhancer associated with a reduced risk of atherosclerosis, cancer, and tissue damage (8). In chicks, vitamin E supplementation alleviated the effects of lipid peroxida-

tion during zinc deficiency (29). Prevention of vitamin E loss may delay development of debilitating diseases and conditions directly affected by retrovirus infection. Low vitamin E levels were seen in patients infected with HIV (30). Prevention of lipid peroxidation of cell membrane by vitamin E is part of its immune enhancing response (31). The rapidly proliferating cells of the immune system are particularly susceptible to oxidative damage by free radicals. The antioxidants also modulate the biosynthesis and activity of important cell regulators, prostaglandin, thromboxanes, and leukotrienes (32).

In the current studies, there was evidence of increased hepatic and cardiac lipid peroxidation in retrovirus infected mice concomitantly with decreased hepatic and cardiac vitamin E levels. This may be due to immune dysfunction induced by the retrovirus altering cytokine production toward that seen in inflammatory diseases (5), which should increase oxidative stress and decrease tissue antioxidant levels. Similarly, plasma lipid peroxidation was increased in AIDS patients (33). Oxidative stress may be a second messenger as TNF-α levels are elevated in the serum of HIV-infected patients (34) and murine AIDS (35). TNF-α and IL-6, produced in excessive quantities during murine and human AIDS, are potent enhancing factors in the spreading of HIV to new target cells (22). Vitamin E supplementation restored a less oxidative environment in murine retrovirus–infected mice while decreasing the excessive IL-6 production (5), much as occurred with TCR peptide therapy which simultaneously prevented vitamin E losses.

Free radicals can induce the expression of HIV in human T cell lines by activating transcription of NF-κB (36). Vitamin E may block NF-κB activation by reducing oxidative stress and IL-6 levels, thereby inhibiting HIV replication and retarding progression of infection. A unique feature of HIV infection is its persistence in a quiescent state, prior to activation, without production of either viral mRNA or proteins. As free radical stimulus seems important to HIV multiplication, vitamin E may retard murine retrovirus replication by lowering the oxidative stress, keeping retrovirus in a quiescent state, and inhibiting progression to murine AIDS. Reduced levels of antioxidants, vitamin E, glutathione, and other acid soluble thiols correlated well with the accelerated progression to human AIDS (37). Oxidative stress may also be a potent inducer of viral activation by causing DNA damage in infected cells, inducing certain alterations in the cells necessary for HIV reproduction, and producing a long-term consequence of HIV infection, immunosuppression (38). If there were reduced levels of superoxide dismutase in early murine retrovirus infection (39), it should result in increased persistence of hydrogen peroxide with more oxidative damage, lipid peroxidation, and loss of vitamin E *via* its reaction with free radicals. The evidence of increased free radical or oxidative activity and greater lipid peroxidation products fit well with a loss of tissue vitamin E during T cell immune dysfunction in murine AIDS. Our data in these studies further support this concept with prevention of immune dysfunction by immunological regulation. TCR peptide treatment largely prevented loss of vitamin E. Low doses or delayed treatment of a TCR peptide Vβ8.1 did not correct the immune dysfunction nor prevented loss of vitamin E (11). T and B cell dysfunction should permit greater bacterial infections, yielding more bacterial lipopolysaccharides for macrophage activation. This would cause release of highly reactive free radicals, altering cellular function and enzymic activity (40). As we have found that vitamin E supplementation partial normalized the immune functions during murine AIDS, tissue vitamin E levels appear to be a critical component in maintaining immune functions against free radical damage. Vitamin E prevented much of the oxidative damage of alcohol alone and during retrovirus infection, retarding esophageal tumor growth induced by a carcinogen (41). Therefore, antioxidant activity is important in preventing tumor growth in murine AIDS, perhaps by immune modulation *via* maintenance of tissue vitamin E (40).

TCR peptides that were effective in preventing immune dysfunction in murine AIDS prevented excessive lipid peroxidation and loss of vitamin E. However, the TCR peptide fragments that did not prevent immune dysfunction also did not prevent oxidative damage.

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