Biomed Pharmacother 2001 ; 55 : 23-31

S0753332200000184/FLA

Dossier: AIDS

Autoantibodies to TNFα in HIV-1 infection: prospects for anti-cytokine vaccine therapy

C.J. Capini[1], M.W. Richardson[1], H. Hendel[2], A. Sverstiuk[1], J. Mirchandani[1], E.G. Régulier[1], K. Khalili[1], J.F. Zagury[2], J. Rappaport[1]*

[1]*Center for Neurovirology and Cancer Biology, 1900 N. 12th St., Rm 246, Temple University, Philadelphia, PA 19122 USA;* [2]*Cellular Biology Laboratory, Pierre and Marie Curie University, Paris, France*

(Received 3 November 2000; accepted 7 November 2000)

Summary – Tumor necrosis factor alpha (TNFα) is a proinflammatory cytokine principally involved in the activation of lymphocytes in response to viral infection. TNFα also stimulates the production of other cytokines, activates NK cells and potentiates cell death and/or lysis in certain models of viral infection. Although TNFα might be expected to be a protective component of an antiviral immune response, several lines of evidence suggest that TNFα and other virally-induced cytokines actually may contribute to the pathogenesis of HIV infection. Based on the activation of HIV replication in response to TNFα, HIV appears to have evolved to take advantage of host cytokine activation pathways. Antibodies to TNFα are present in the serum of normal individuals as well as in certain autoimmune disorders, and may modulate disease progression in the setting of HIV infection. We examined TNFα-specific antibodies in HIV-infected non-progressors and healthy seronegatives; anti-TNFα antibody levels are significantly higher in GRIV seropositive slow/non-progressors (N = 120, mean = 0.24), compared to seronegative controls (N = 12, mean = 0.11). TNFα antibodies correlated positively with viral load, (P = 0.013, r = 0.282), and CD8+ cell count (P = 0.03, r = 0.258), and inversely with CD4+ cell count (P = 0.003, r = – 0.246), percent CD4+ cells (P = 0.008, r = –0.306), and CD4 :CD8 ratio (P = 0.033, r = – 0.251). TNFα antibodies also correlated positively with antibodies to peptides corresponding to the CD4 binding site of gp160 (P = 0.001, r = 0.384), the CD4 identity region (P = 0.016, r = 0.29), the V3 loop (P = 0.005, r = 0.34), and the amino terminus of Tat (P = 0.001, r = 0.395); TNFα antibodies also correlated positively with antibodies to Nef protein (P = 0.008, r = 0.302). The production of anti-TNFα antibodies appears to be an adaptive response to HIV infection and suggests the potential utility of modified cytokine vaccines in the treatment of HIV infections as well as AIDS-related and unrelated autoimmune and CNS disorders. © 2001 Éditions scientifiques et médicales Elsevier SAS

AIDS-vaccine / autoantibodies / HIV / tumor necrosis factor alpha

In targeting immune cells for viral infection, HIV has evolved regulatory mechanisms that permit rapid deployment in response to immune activation signals. The same pathways that induce T cell proliferation and macrophage activation are usurped by HIV to activate viral replication [1-6]. TNFα is a potent proinflammatory cytokine integral to antiviral immune responses, which induces apoptosis of virally-infected cells and contributes to cytotoxic T cell-mediated lysis and lymphocyte and monocyte differentiation [7]. Interaction of TNFα with cell surface receptors can transduce a variety of intracellular signals via phospholipase A2, protein kinase C

*Correspondence and reprints.
E-mail address: jayrapp@astro.temple.edu (J. Rappaport).

and activation of several transcription factors including NFKB [8]. It is well established that the major enhancer element within the HIV-1 LTR promoter is comprised of two NFKB binding sites responsive to T cell activation and cytokine activation signals [1-6]. These same transcriptional elements are involved in IL-2 and IL-2R gene expression [3-6]. TNFα production results in coordinate activation of certain inflammatory cytokines, including chemokines, via autocrine and paracrine mechanisms [9-12]. TNFα production results in alteration of endothelial cell monocyte/macrophage interactions, leading to invasion of inflammatory cells into various tissues including the central nervous system [12-14]. This process appears to be a critical pathway in the development of HIV-associated neurologic disorders, with increased accumulation of activated monocytes/macrophages in the CNS compartment.

It is well established that healthy individuals as well as patients with autoimmune disorders produce natural autoantibodies against TNFα [15]. Anti-cytokine autoantibodies (i.e. anti-TNFα and interferon-γ autoantibodies) appear to play a regulatory role in the immune response [16], and may serve to counteract the potential deleterious effects of these inflammatory mediators in vivo. In order to evaluate the significance of anti-TNFα autoantibodies in HIV infection, we produced recombinant human TNFα as a fusion protein, assayed serum samples from HIV infected slow/non-progressors and seronegatives in ELISA, and correlated levels of antibodies to TNFα with serologic and hematologic evaluation parameters.

MATERIALS AND METHODS

Production of the recombinant tumor necrosis factor α

The cDNA of the mature hTNFα was obtained by PCR amplification from the plasmid pORF-hTNFα (Invivogen, San Diego, CA), cloned in the prokaryote expression vector pRSET-A (Invitrogen, San Diego, CA), and confirmed by automated DNA sequencing. This construct permits expression of TNFα as a fusion-protein with an N-terminal polyhistidine-tag, thereby permitting purification by metal chelate affinity chromatography. *Figure 1* illustrates the amino acid sequence of the hTNFα fusion-protein.

^{1}*MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDRW* ^{34}
^{35}*GSELE*VRSSSRTPSDKPVAHVVANPQAEGQLQWLN ^{69}
^{70}RRANALLANGVELRDNQLVVPSEGLYLIYSQVLFK ^{104}
^{105}GQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPC ^{140}
^{141}QRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEI ^{175}
^{176}NRPDYLDFAESGQVYFGIIAL ^{196}

Molecular weight: 22 kDa
Estimated pI: 6.14

Figure 1. Amino acid sequence of the hTNFα_r fusion protein. The fusion domain containing the polyhistidine tag is in italics.

The plasmid pRSETA-TNFα was transformed into *E. coli* (BL21-DE3 CodonPlus, Stratagene, San Diego, CA) for protein expression. Bacteria were grown in one liter of 2X LB media. TNFα fusion protein expression was induced at O.D. 0.6–0.8 with 1 mM IPTG (Calbiochem, San Diego, CA), and grown overnight at 37° C and 250 rpm. The culture (O.D. 1.4) was collected by centrifugation. Pellets were resuspended in 50 mL of Buffer A (300 mM NaCl, 50 mM Hepes, pH 7.2, 0.025% Octyl-β-Glucoside and CHAPS). The cells were disrupted by sonication using a Branson Sonifier (3X) for 30 s with 5 min on ice between sonication intervals. Homogenate was centrifuged at 14,000 rpm (30,000 *g*) for 15 min; supernatant was applied to a 5 mL Talon® Metal Affinity Resin column (Clontech, Palo Alto, CA), and packed in an XK16/20 column (Amersham-Pharmacia, Piscataway, NJ). The column was washed with 10 column volumes of Buffer A, followed by 4 column volumes of Buffer A containing 10 mM imidazole, and 6 column volumes of Buffer A containing 50 mM imidazole. Elution was performed with 5 column volumes of Buffer A containing 500 mM imidazole. Chromatography was performed throughout at a flow rate of 1 mL/min using an Acuflow Series III pump (LabAlliance, State College, PA). Affinity purified TNFα was collected in 20 mL fractions and protein containing fractions were pooled. The sample (15 mL; 2.6 mg/mL) was diafiltered under nitrogen with five volumes of Buffer B (300 mM NaCl, 50 mM Hepes, pH 7.2) using an Amicon 8050 stirred cell with a 10,000 NML 45 mm polyethersulfone membrane (Millipore, Bedford, MA) pretreated with 5% polyethylene glycol reacted with Bisphenol A

(Sigma-Aldrich, Steinheim, Germany). The final preparation (30 mL; 0.8 mg/mL.) was stored in aliquots at – 80° C.

Patient samples

Serum from HIV-1-infected non-progressors (N = 120) were compared with samples from seronegative controls (N = 12) for autoantibodies to TNFα. Slow/non-progressor (S/NP) volunteers were previously enrolled in the genetic resistance to immunodeficiency virus (GRIV) cohort to study genetic and serologic parameters associated with non-progression. This cohort, established in France, has been useful in identifying genetic polymorphisms and serologic responses associated with non-progression in the setting of HIV infection [17-22]. The inclusion criteria for S/NPs were as follows: seropositive for HIV-1 and asymptomatic for more than eight years, with a CD4+ cell count greater than 500/mm^3 without prior antiretroviral therapy. All seropositives in this group were Caucasians for the purpose of simplifying genetic analysis. Serum samples from HIV-negative individuals were collected at MCP-Hahnemann University (Philadelphia, PA) with informed consent. All seronegatives used in this study were Caucasians for comparison with the GRIV S/NP samples.

ELISA assay for TNFα autoantibodies

The recombinant human TNFα, 0.5 µg per well diluted in 100 µL of 50 mM $NaHCO_3$, pH 9.0, was immmobilized in Maxisorb™ microtiter plates (Nunc, Rochester, NY) by overnight incubation at 4° C. Plates were blocked for three hours at room temperature with 250 µL/well 1X PBS containing 3% IgG-free BSA (Sigma, St. Louis, MO). Plates were washed 6X in 1X PBS with 0.05% Tween 20 using an Ultrawash Plus plate washer (Dynex, Chantilly, VA). Diluted sera (1:500, 50 µL per well) were incubated overnight at 4° C while shaking. Plates were washed as before and Protein G-HRP (50 mL, 1:1000 dilution; Biorad, Hercules, CA) was added and incubated for 2 h at room temperature while shaking. Plates were washed again as before and developed by addition of TMBlue (Intergen, Milford, MA), 50µL per well. The reaction was stopped after 5 min by the addition of 50 µL 2N HCl, and plates were read at $OD_{\lambda 450}$ on an MRX microplate reader with Revelation software (Dynex, Chantilly, VA).

Statistical analysis

Statistical analysis, including ANOVA and both parametric and nonparametric correlations, was performed using SPSS 10.0 software for Windows (SPSS Inc., Chicago, IL). Briefly, TNFα antibodies in both GRIV S/NP and seronegative controls were determined to be normally distributed using the Kolmogorov-Smirnov test for normality; ANOVA was performed to test for significant differences between TNFα antibody levels of GRIV S/NP relative to seronegative controls. TNFα antibody levels in GRIV S/NP were correlated with peripheral blood lymphocyte and T cell subset counts, antibody responses to viral proteins including p24, Tat and Nef, and both Tat and gp120-derived peptides. Antibodies to peptide 34, as well as CD4 and CD8 cell counts, and the percent CD4 positive cells were determined to be normally distributed by Kolmogorov-Smirnov. In contrast, viral load, CD4:CD8 ratio, and antibodies to peptides 32, 135, 164, 243, 356, 385, and Tat and Nef fusion proteins were not normally distributed. Correlations between TNFα antibodies and viral load, CD4 count, CD4 percent, CD4:CD8 ratio, antibodies to peptides 32, 34, 243, 356, and Nef protein were significant using both parametric and nonparametric tests; Pearson's coefficients and P-values are reported in the text. Although the correlation between TNFα antibodies and CD8 cell count only passed the parametric Pearson procedure, it was deemed significant because both sets of data were normally distributed. Similarly, although correlations between antibodies to TNFα and peptides 135 and 385 only passed the non-parametric Kendall and Spearman procedures, they were deemed significant because antibodies to both peptides 135 and 385 were not normally distributed; Spearman's coefficients and P-values were reported in the text.

RESULTS AND DISCUSSION

The T cell depletion characteristic in AIDS may be due in part to TNFα dysregulation; TNFα secretion contributes to wasting and T cell anergy, and can provide a pro-apoptotic signal for both CD4+ and CD8+ T cells. TNFα also has been implicated in HIV encephalophathy and HIV-associated progressive

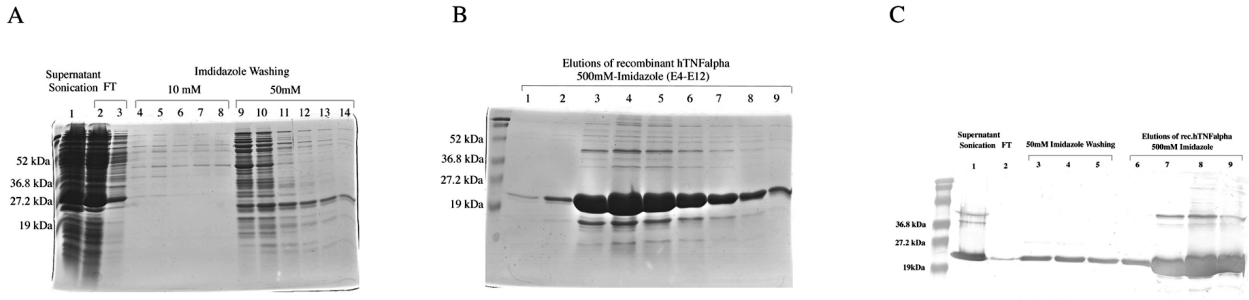

Figure 2. Expression and purification of hTNFα fusion protein under native conditions using metal chelate affinity chromatography. A) Coomassie stain of 14% SDS-PAGE of 5 mL Talon column load, flow-through, and 10 mM and 50 mM Imidazole washes. B) Coomassie stain of 14% SDS-PAGE of 500 mM Imidazole elution. The main band is a monomer of approximately 22 kDa; the less intense band of approximately 45–55 kDa is presumably the TNFα trimer. C) DAB-stained Western blot of 14% SDS-PAGE of load, flow-through, 50 mM Imidazole wash, and 500 mM Imidazole elution using anti-HisG-HRP monoclonal antibody at 1:5000 dilution (Invitrogen, San Diego, CA).

multifocal leukoencephalopathy, as well as other CNS disorders [12-14, 23-27]. In HIV infection, TNFα production is elevated as demonstrated by leukocyte TNFα mRNA levels, as well as membrane-bound and circulating forms of TNFα [28-32]. Circulating soluble TNFα receptor is also elevated and most likely antagonizes the effects of circulating and membrane-bound TNFα [28, 30, 33, 34]. The importance of activation of TNFα synthesis in the pathogenesis of HIV infection has been further emphasized by studies demonstrating reduced TNFα production with successful application of highly aggressive antiretroviral therapy (HAART) [35-38].

HIV infection increases TNFα gene expression by interaction of the envelope glycoprotein with CD4 receptors and coreceptors [39-41], as well as by Tat-mediated activation [14, 42, 43]. TNFα in turn exerts complex effects on T cells, such as stimulating proliferation, and inducing or preventing apoptosis [44]. HIV-induced TNFα dysregulation is involved in apoptosis of CD4+ cells through a process apparently enhanced by TNFα-mediated upregulation of Fas ligand [45-47]. TNFα activation is also involved in CD8+ cell apoptosis in AIDS through interaction of membrane-bound TNFα on macrophages and TNF receptors on CD8+ cells [48]. TNFα also appears to contribute to polyclonal B cell proliferation in HIV infection [49, 50]. Although TNFα increases HIV replication at the level of transcription, TNFα may actually suppress CCR5 tropic virus infection via induction of β chemokines and inhibition of CCR5 gene expression, as demonstrated with the monocyte-tropic NSI virus [9]. In contrast, TNFα upregulates the CXCR4 receptor on T cells, increasing the susceptibility to infection with cytopathic, syncytia-inducing (SI) viruses [51]. In view of the role of SI variants in the pathogenesis of HIV infection, TNFα may play a critical role in viral evolution toward pathogenic variants. Based on the functional properties of TNFα in facilitating HIV viral replication, evolution of pathogenic variants and dysregulation of the immune system, autoantibodies to TNFα may be an advantageous host response to viral infection and chronic TNFα upregulation.

In order to study the levels of TNFα autoantibodies, we expressed and purified recombinant human TNFα for use in ELISA for autoantibodies in serum from HIV-positive volunteers. TNFα was expressed in soluble form in *E. coli* after overnight induction with IPTG. From one liter of culture, 24 mg of recombinant protein (rhTNFα) was purified by affinity chromatography using Talon resin (Qiagen, Valencia, CA). *Figure 2* illustrates the protein profiles of the extract and purified fractions obtained during the purification process. A major band of 22 kDa was visualized in agreement with the predicted migration of the hTNFα fusion protein. The slower-migrating band presumably represents the TNFα trimer (45–55 kDa); the trimer represents the active form of this protein in solution [52]. Antigenicity of the unmodified fusion protein was confirmed by Western blot *(figure 2)*, and in ELISA (data not shown) using goat anti-TNFα antibody (R & D Systems, Minneapolis, MN).

The purified rhTNFα was used in ELISA to determine the level of autoantibodies from HIV-infected

S/NPs and controls. As shown in *figure 3*, the level of autoantibodies to TNFα was significantly higher ($P = 0.005$; ANOVA) in the seropositive group ($N = 120$, mean = 0.244), relative to the seronegative control group ($N = 12$, mean = 0.114). Among S/NPs, autoantibodies to TNFα positively correlated with viral load ($P = 0.013$, Pearson = 0.282), and with CD8+ cell number ($P = 0.03$, Pearson = 0.258). Autoantibodies to TNFα correlated inversely with CD4+ cell number ($P = 0.003$, Pearson = –0.246), percent CD4+ ($P = 0.008$, Pearson = – 0.306). No significant correlation with age, sex, or total lymphocyte count was observed. Correlation of anti-TNFα antibodies with other antibody responses to Tat and gp160 peptides are shown in *table I* [53-59]. TNFα antibodies positively correlated with antibodies to gp160 (a.a. 418–444) peptide 32 ($P = 0.0001$, Pearson = 0.467), and peptide 34 ($P = 0.001$, Pearson = 0.384), both corresponding to the CD4 binding site. Correlations were also observed with antibodies to peptide 243 containing the 'SLWDQ' CD4 identity region of gp120 ($P = 0.016$, Pearson = 0.29), and peptide 356 containing a reiterated SLWDQ sequence ($P = 0.0001$, Pearson = 0.429), as well as peptide 135 ($P = 0.005$, Spearman = 0.34), encoding the V3 loop (a.a 290–323). There was no correlation with antibodies to several other regions of gp160 (peptides 163, 12, and 23), as illustrated in *table I*. However, as shown in *table I*, a correlation between TNFα antibodies and anti-Nef antibodies was observed ($P = 0.008$, Pearson = 0.302). There was no correlation between TNFα antibodies and antibodies to recombinant N-terminal polyhistidine fusion Tat protein. Interestingly, a correlation was observed with antibodies to the amino-terminal region of Tat ($P = 0.001$, Spearman = 0.395), using peptide 385 (a.a. 1–15 of Tat).

Anti-TNFα antibodies may be a natural response to excess TNFα generated as a result of HIV infection. These autoantibodies may serve to mollify the harmful effects of TNFα continually produced by virally-infected cells. The effect of TNFα in activating HIV replication at the level of transcription is well established via the NFKB pathway [2, 59, 60]. It appears that in contrast to the usual antiviral function of TNFα, this cytokine pathway is activated by viral infection and utilized to promote viral infection and injury to the immune system and CNS. The release of soluble TNF receptors in circulation is evident in HIV infection and may provide one means of reducing the harmful effects of TNFα activation. Here we demonstrate the presence of autoantibodies to TNFα in HIV-infected S/NPs, which may provide an additional adaptive response to TNFα dysregulation.

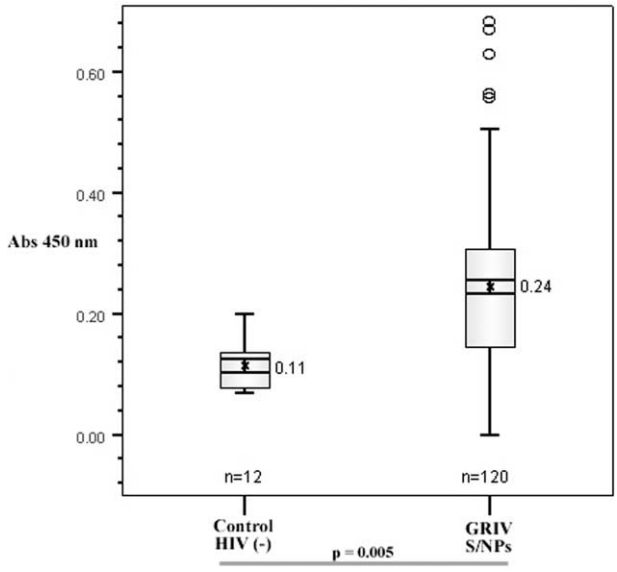

Figure 3. Box-plot of results from ELISA for serum antibodies (IgG) to TNFα. Anti-TNFα antibodies are significantly elevated ($P = 0.005$, ANOVA), in GRIV S/NPs ($N = 120$, mean = 0.24), compared to seronegative controls ($N = 12$, mean = 0.11). The mean OD of each group is indicated in the box-plot with a cross; the numerical value is also given. Dark horizontal lines connected by a vertical line within each box represent the standard error of the mean for each group. The upper and lower boundaries of the boxes represent the 75th and 25th percentiles, respectively. Whiskers represent the range of values observed in each group. Outliers are indicated with circles.

In view of the involvement of TNFα in virus replication and pathogenesis, the development of a vaccine designed to elicit antibodies to TNFα may help restore immune function and at the same time reduce viral replication. Aside from the potential utility of a vaccine to TNFα in HIV infection, this approach may have utility in a variety of clinical disorders where cytokine imbalance contributes substantially to the disease process. For example, in hairy cell leukemia, TNFα may be important for hairy cell leukemia growth [61, 62]. TNFα has been implicated in the pathogenesis of Crohn's disease and other inflammatory bowel diseases and is the target of two therapies: etanercept, a soluble TNF receptor linked to the Fc portion of human IgG1 [63], and infliximab, a

Table I. Synthetic HIV polypeptides used to detect antibody titers in the GRIV non-progressor population.

HXB2 position	*Peptide number*	*Amino acid sequence*	*Source*	*Presumptive role*	*Correlation TNFα autoantibodies*					
					Pearson		*Kendall*		*Spearman*	
					Coeff.	P-*value*	*Coeff.*	P-*value*	*Coeff.*	P-*value*
$gp160_{294-321}$	12	INCTRPNYNKRKRIHGPGRAFYTTK	MN isolate [53]	Env V_3 loop fragment	0.14	0.259	0.075	0.369	0.116	0.348
$gp160_{584-604}$	23	ERYLKDQQLLGIWGCSGKLIC	BRU isolate [54]	C-terminal fragment of gp120	0.14	0.256	0.071	0.391	0.115	0.349
$gp160_{418-444}$	32	CRIKQIINMWQGVGKAMYAPPIEGQIN	Z6 isolate [55]	CD4 binding site of gp120	0.467	0.0001	0.312	0.0001	0.438	0.0001
$gp160_{418-444}$	34	CRIKQIINMWQEVGKAMYAPPISGQIR	BH8 isolate [56]	CD4 binding site of gp120	0.384	0.001	0.263	0.002	0.391	0.007
$gp160_{290-323}$	135	FLLAVFCTRPNYNKRKIHIGPGRAFYT-TKNIIG	MN isolate	Env V_3 loop fragment	0.148	0.234	0.24	0.004	0.34	0.005
$gp160_{837-856}$	163	CRAIRHIPRRIRQGLERILL	BRU isolate	C-terminal fragment of gp41	0.143	0.244	0.081	0.33	0.096	0.437
$gp160_{110-125}$	243	SLWDQSLKPCVKLTPL	MN isolate	CD4 identity of gp120 [57]	0.29	0.016	0.221	0.008	0.315	0.009
$gp160_{110-125}$ (reiterated)	356	SLWDQSLWDQSLWDQSLWDQ	MN isolate	Reiterated SLWDQ fragment of gp160 CD4 identity	0.429	0.0001	0.0367	0.0001	0.547	0.0001
$gp160_{289-305}$	362	NQSVEINCTRPNNNTRK	BRU isolate	*Fas* identity of gp120	0.119	0.335	0.085	0.307	0.116	0.345
Tat_{1-15}	385	MEPVDPRLEPWKHPG	MN isolate	N-terminal fragment of transactivating regulatory protein	0.157	0.2	0.283	0.001	0.395	0.001
Tat		Fusion protein	III B	Transactivator	0.006	0.96	0.012	0.881	0.018	0.879
Nef	Fusion protein		pSVL4-3	CD4 downregulation and pathogenesis	0.302	0.008	0.245	0.002	0.344	0.002

humanized monoclonal antibody [64, 65]. TNFα has also been implicated in the pathogenesis of rheumatoid arthritis [66], sarcoidosis [67], sepsis and myelodysplastic syndromes [68].

The use of anti-cytokine vaccines in the form of 'kinoids' has been previously proposed [69, 70]. This strategy has provided encouraging results in a murine model of experimental cachexia and type II collagen-induced arthritis using a chimeric TNFα vaccine containing immunodominant T_h epitopes [71]. The application of TNFα vaccine strategies may provide a therapeutic approach to the treatment of HIV infection as well as a wide variety of clinical disorders.

CONCLUSIONS

We produced and purified a recombinant TNFα fusion protein to detect autoantibodies to TNFα in HIV-infected slow/non-progressors (S/NPs). Anti-TNFα antibodies are significantly elevated in HIV-infected non-progressors, who are naïve to antiretroviral therapy, compared to seronegatives. Because TNFα activates viral replication and induces immune dysregulation, the ability of the host to interfere with chronic TNFα upregulation may be important for prevention of disease progression. Vaccine strategies designed to boost these natural anti-cytokine autoantibodies comprise important therapeutic venues for AIDS treatment. Further studies will be required to determine if anti-TNFα antibodies are indeed a beneficial host response and to evaluate the concept of a TNFα-targeted vaccine.

ACKNOWLEDGEMENTS

We would like to acknowledge the support from Medivacs, Inc. (Bala Cynwyd, PA) to JR, from Neovacs, Inc. to JFZ, and from NIH grants to JR and KK.

REFERENCES

1 Green WC, Bohnlein E, Ballard DW. HIV-1, HTLV-1 and normal T-cell growth: transcriptional strategies and surprises. Immunol Today 1989 ; 10 : 272-8.

2 Griffin GE, Leung K, Folks TM, Kunkel S, Nabel GJ. Activation of HIV gene expression during monocyte differentiation by induction of NF-kappa B. Nature 1989 ; 339 : 70-3.

3 Cross SL, Halden NF, Lenardo MJ, Lenardo WJ. Functionally distinct NF-kappa B binding sites in the immunoglobulin kappa and IL-2 receptor alpha chain genes. Science 1989 ; 244 : 466-9.

4 Hoyos B, Ballard DW, Bohnlein E, Siekevitz M, Green WC. Kappa B-specific DNA binding proteins: role in the regulation of human interleukin-2 gene expression. Science 1989 ; 244 : 457-60.

5 Lownthal JW, Ballard DW, Bohnlein E, Green WC. Tumor necrosis factor alpha induces proteins that bind specifically to kappa B-like enhancer elements and regulate interleukin 2 receptor alpha-chain gene expression in primary human T lymphocytes. Proc Natl Acad Sci USA 1989 ; 86 : 2331-5.

6 Tong-Starkesen SE, Luciw PA, Peterlin BM. Functionally distinct NF-kappa B binding sites in the immunoglobulin kappa and IL-2 receptor alpha chain genes. Science 1989 ; 244 : 466-9.

7 Smith MJ, Johnstone RW. Role of TNF in lymphocyte-mediated cytotoxicity. Microsc Res Tech 2000 ; 50 : 196-208.

8 Schutze S, Machleidt T, Kronke M. Mechanisms of tumor necrosis factor action. Semin Oncol 1992 ; 19 : 16-24.

9 Hornung F, Scala G, Lenardo MJ. TNF-alpha-induced secretion of C-C chemokines modulates C-C chemokine receptor 5 expression on peripheral blood lymphocytes. J Immunol 2000 ; 64 : 6180-7.

10 Kunsch C, Rosen CA. F-kappa B subunit-specific regulation of the interleukin-8 promoter. Mol Cell Biol 1993 ; 13 : 6137-46.

11 Moriuchi H, Moriuchi M, Fauci AS. Nuclear factor-kappa B potently up-regulates the promoter activity of RANTES, a chemokine that blocks HIV infection. J Immunol 1997 ; 158 : 3483-91.

12 Schmidtmayerova H, Nottet HS, Nuovo G, Raabe T, Flanagan CR, Dubrovsky L, et al. Human immunodeficiency virus type 1 infection alters chemokine beta peptide expression in human monocytes: implications for recruitment of leukocytes into brain and lymph nodes. Proc Natl Acad Sci U S A 1996 ; 93 : 700-4.

13 Bonwetsch R, Croul S, Richardson MW, Lorenzana C, Valle LD, Sverstiuk AE, et al. Role of HIV-1 Tat and CC chemokine MIP-1alpha in the pathogenesis of HIV associated central nervous system disorders. J Neurovirol 1999 ; 5 : 685-94.

14 Rappaport J, Joseph J, Croul S, Alexander G, Del Valle L, Amini S, et al. Molecular pathway involved in HIV-1-induced CNS pathology: role of viral regulatory protein. Tat. J Leukoc Biol 1999 ; 65 : 458-65.

15 Sioud M, Dybwad A, Jespersen L, Suleyman S, Natvig JB, Forre O. Characterization of naturally occurring autoantibodies against tumour necrosis factor-alpha (TNF-alpha): in vitro function and precise epitope mapping by phage epitope library. Clin Exp Immunol 1994 ; 98 : 520-5.

16 Bakhiet M, Elkarim R, Diab A, Zhu J, Lindquist L, Link H, et al. A novel mechanism for cytokine regulation: screening, selection, and characterization of anticytokine monoclonal and polyclonal autoantibodies. J Interferon Cytokine Res 1999 ; 19 : 439-45.

17 Rappaport J, Cho YY, Hendel H, Schwartz EJ, Schachter F, Zagary JF. 32 bp CCR-5 gene deletion and resistance to fast progression in HIV-1 infected heterozygotes. Lancet 1997 ; 349 : 922-3.

18 Hendel H, Cho YY, Gauthier N, Rappaport J, Schachter F, Zagury JF. Contribution of cohort studies in understanding HIV pathogeneis: introduction of the GRIV cohort and preliminary results. Biomed Pharmacother 1996 ; 50 : 480-7.

19 Zagury JF, Abderrahim Lachgar A, Le Buanec H, Richardson M, Rappaport J, Hendel H, et al. Antibodies to the HIV-1 Tat protein correlate with non-progression to AIDS: a rationale for the use of a Tat toxoid as an HIV-1 vaccine. J Hum Virol 1998 ; 1 : 282-92.

20 Hendon H, Henon N, Lebuanec H, Lachgar A, Poncelet H, Caillat-Zuoman S, et al. Distinctive effects of CCR5, CCR2 and SDF-1 genetic polymorphisms in AIDS progression. J Acquir Immune Defic Syndr Hum Retrovirol 1998 ; 19 : 381-6.

21 Hendel H, Caillat-Zucman S, Lebuanec H, Carrington M, O'Brien S, Andrieu JM, et al. New class I and II alleles strongly associated with opposite patterns of progression to AIDS. J Immunology 1999 ; 162 : 6942-6.
22 Richardson MW, Sverstiuk A, Hendel H, Cheung TW, Zagury JF, Rappaport J. Analysis of telomere length and thymic output in fast and slow/non-progressors with HIV infection. Biomed Pharmacother 2000 ; 54 : 21-31.
23 Probert L, Akassoglou K, Kassiotis G, Pasparakis M, Alexopoulou L, Kollias G. TNF-alpha transgenic and knockout models of CNS inflammation and degeneration. J Neuroimmunol 1997 ; 72 : 137-41.
24 Tyor WR, Wesselingh SL, Griffin JW, McArthur JC, Griffin DE. Unifying hypothesis for the pathogenesis of HIV-associated dementia complex, vacuolar myelopathy and sensory neuropathy. J Acquir Immune Defic Syndr Hum Retrovirol 1995 ; 9 : 379-88.
25 Hartung HP, Jung S, Stoll G, Zielasek J, Schmidt B, Archelos JJ, et al. Inflammatory mediators in demyelinating disorders of the CNS and PNS. J Neuroimmunol 1992 ; 40 : 197-210.
26 Grimaldi LM, Martino GV, Franciotta DM, Brustia R, Castagna A, Pristera R, et al. Elevated alpha-tumor necrosis factor levels in spinal fluid from HIV-1-infected patients with central nervous system involvement. Ann Neurol 1991 ; 29 : 21-5.
27 Mastroianni CM, Paoletti F, Massetti AP, Falciano M, Vullo V. Elevated levels of tumor necrosis factor (TNF) in the cerebrospinal fluid from patients with HIV associated neurological disorders. Acta Neurol (Napoli) 1990 ; 12 : 66-7.
28 Fahey JL, Taylor JM, Manna B, Nishanian P, Aziz N, Giorgi JV, et al. Prognostic significance of plasma markers of immune activation, HIV viral load and CD4 T-cell measurements. AIDS 1998 ; 12 : 1581-90.
29 Benyoucef S, Hober D, Shen L, Ajana F, De Groote D, Bocket-Mouton L, et al. Production of TNF alpha and IL-6 by activated whole blood from HIV-1 infected patients detected by a one-stage procedure: relationship with the phenotype of HIV-1 isolates. Microbiol Immunol 1997 ; 41 : 939-46.
30 Hestdal K, Aukrust P, Muller F, Lien E, Bjerkeli V, Espevik T, et al. Dysregulation of membrane-bound tumor necrosis factor-alpha and tumor necrosis factor receptors on mononuclear cells in human immunodeficiency virus type 1 infection: low percentage of p75-tumor necrosis factor receptor positive cells in patients with advanced disease and high viral load. Blood 1997 ; 90 : 2670-9.
31 Scott-Algara D, Vuillier F, Marasescu M, de Saint Martin J, Dighiero G. Serum levels of IL-2, IL-1 alpha, TNF-alpha, and soluble receptor of IL-2 in HIV-1-infected patients. AIDS Res Hum Retrovir 1991 ; 7 : 381-6.
32 Altfeld M, Addo MM, Kreuzer KA, Rockstroh JK, Dumoulin FL, Schliefer K, et al. T(H)1 to T(H)2 shift of cytokines in peripheral blood of HIV-infected patients is detectable by reverse transcriptase polymerase chain reaction but not by enzyme-linked immunosorbent assay under nonstimulated conditions. J Acquir Immune Defic Syndr Hum Retroviral 2000 ; 23 : 287-94.
33 Bilello JA, Stellrecht K, Drusano GL, Stein DS. Soluble tumor necrosis factor-alpha receptor type II (sTNF alpha RII) correlates with human immunodeficiency virus (HIV) RNA copy number in HIV-infected patients. J Infect Dis 1996 ; 173 : 464-7.
34 Hober D, Benyoucef S, Delannoy AS, de Groote D, Ajana F, Mouton Y, et al. High plasma level of soluble tumor necrosis factor receptor type II (sTNFRII) in asymptomatic HIV-1-infected patients. Infection 1996 ; 24 : 213-7.
35 Ledru E, Christeff N, Patey O, de Truchis P, Melchior JC, Gougeon ML. Alteration of tumor necrosis factor-alpha T-cell homeostasis following potent antiretroviral therapy: contribution to the development of human immunodeficiency virus-associated lipodystrophy syndrome. Blood 2000 ; 95 : 3191-8.
36 Aukrust P, Muller F, Lien E, Nordoy I, Liabakk NB, Kvale D, et al. Tumor necrosis factor (TNF) system levels in human immunodeficiency virus-infected patients during highly active antiretroviral therapy: persistent TNF activation is associated with virologic and immunologic treatment failure. J Infect Dis 1999 ; 179 : 74-82.
37 Gendelman HE, Zheng J, Coulter CL, Ghorpade A, Che M, Thylin M, et al. Suppression of inflammatory neurotoxins by highly active antiretroviral therapy in human immunodeficiency virus-associated dementia. J Infect Dis 1998 ; 178 : 1000-7.
38 Chun TW, Engel D, Mizell SB, Ehler LA, Fauci AS. Induction of HIV-1 replication in latently infected CD4+ T cells using a combination of cytokines. J Exp Med 1998 ; 188 : 83-91.
39 Zembala M, Pryjma J, Plucienniczak A, Szczepanek A, Ruggiero I, Jasinski M, et al. Modulation of antigen-presenting capacity of human monocytes by HIV-1 GP120 molecule fragments. Immunol Invest 1994 ; 23 : 189-99.
40 Rimaniol AC, Boussin FD, Dormont D, Bach JF, Zavala F. Mechanisms of downmodulation and release of tumour necrosis factor receptor induced by human immunodeficiency virus type 1 in human monocytes. Cytokine 1997 ; 9 : 9-18.
41 Tamma SM, Chirmule N, Yagura H, Oyaizu N, Kalyanaraman V, Pahwa S. CD4 cross-linking (CD4XL) induces RAS activation and tumor necrosis factor-alpha secretion in CD4+ T cells. Blood 1997 ; 90 : 1588-93.
42 Sawaya BE, Thatikunta P, Denisova L, Brady J, Khalili K, Amini S. Regulation of TNFalpha and TGFbeta-1 gene transcription by HIV-1 Tat in CNS cells. J Neuroimmunol 1998 ; 87 : 33-42.
43 Mayne M, Bratanich AC, Chen P, Rana F, Nath A, Power C. HIV-1 tat molecular diversity and induction of TNF-alpha: implications for HIV-induced neurological disease. Neuroimmunomodulation 1998 ; 5 : 184-92.
44 Smyth MJ, Johnstone RW. Role of TNF in lymphocyte-mediated cytotoxicity. Microsc Res Tech 2000 ; 50 : 196-208.
45 Algeciras A, Dockrell DH, Lynch DH, Paya CV. CD4 regulates susceptibility to Fas ligand- and tumor necrosis factor-mediated apoptosis. J Exp Med 1998 ; 187 : 711-20.
46 Badley AD, Dockrell D, Simpson M, Schut R, Lynch DH, Leibson P, et al. Macrophage-dependent apoptosis of CD4+ T lymphocytes from HIV-infected individuals is mediated by FasL and tumor necrosis factor. J Exp Med 1997 ; 185 : 55-64.
47 Oyaizu N, McCloskey TW, Than S, Hu R, Kalyanaraman VS, Pahwa S. Cross-linking of CD4 molecules upregulates Fas antigen expression in lymphocytes by inducing interferon-gamma and tumor necrosis factor-alpha secretion. Blood 1994 ; 84 : 2622-31.
48 Herbein G, Mahlknecht U, Batliwalla F, Gregersen P, Pappas T, Butler J, et al. Apoptosis of CD8+ T cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. Nature 1998 ; 395 : 189-94.
49 Maggi E, Almerigogna F, Del Prete G, Romagnani S. Abnormal B cell helper activity by virus-infected human CD4+ T cells. Semin Immunol 1993 ; 5 : 449-55.
50 Patke CL, Shearer WT. gp120- and TNF-alpha-induced modulation of human B cell function: proliferation, cyclic AMP generation, Ig production, and B-cell receptor expression. J Allergy Clin Immunol 2000 ; 105 : 975-82.
51 Juffermans NP, Paxton WA, Dekkers PE, Verbon A, de Jonge E, Speelman P, et al. Up-regulation of HIV coreceptors CXCR4 and CCR5 on CD4(+) T cells during human endotoxemia and after stimulation with (myco)bacterial antigens: the role of cytokines. Blood 2000 ; 96 : 2649-54.
52 Eck MJ, Sprang SR. The structure of tumor necrosos factor alpha at 2.6 A resolution. Inplications for receptor binding. J Biol Chem 1989 ; 264 : 17595-605.

53 Gurgo C, Guo HG, Franchini G, Aldovini A, Collalti E, Farrell K, et al. Envelope sequences of two new United States HIV-1 isolates. Virology 1988 ; 164 : 531-6.
54 Wain-Hobson S, Sonigo P, Danos O, Cole S, Alison M. Nucleotide sequence of the AIDS virus, LAV. Cell 1985 ; 40 : 9-17.
55 Srinivasan A, Anand R, York D, Ranganathan P, Feorino P, Schochetman G, et al. Molecular characterization of human immunodeficiency virus from Zaire: nucleotide sequence analysis identifies conserved and variable domains in the envelope gene. Gene 1987 ; 52 : 71-82.
56 Ratner L, Haseltine W, Patarca R, Livak KJ, Starcich BR, Josephs SF, et al. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature 1985 ; 313 : 277-84.
57 Zagury JF, Bernard J, Achour A, Astgen A, Lachgar A, Fall L, et al. Identification of CD4 and major histocompatibility complex functional peptide sites and their homology with oligopeptides from human immunodeficiency virus type 1 glycoprotein gp120: role in AIDS pathogenesis. Proc Natl Acad Sci U S A 1993 ; 90 : 7573-7.
58 Szawlowski PW, Hanke T, Randall RE. Sequence homology between HIV-1 gp120 and the apoptosis mediating protein Fas. AIDS 1993 ; 7 : 1018.
59 Israel N, Hazan U, Alcami J, Munier A, Arenzana-Seisdedos F, Bachelerie F, et al. Tumor necrosis factor stimulates transcription of HIV-1 in human T lymphocytes, independently and synergistically with mitogens. J Immunol 1989 ; 143 : 3956-60.
60 Lu YC, Touzjian N, Stenzel M, Dorfman T, Sodroski JG, Haseltine WA. The NF kappa B independent cis-acting sequences in HIV-1 LTR responsive to T-cell activation. J Acquir Immune Defic Syndr Hum Retrovirol 1991 ; 4 : 173-7.
61 Aderka D, Levo Y, Ramot B, Michalevicz R, Meytes D, Shaklai M, et al. Reduced production of tumor necrosis factor by mononuclear cells in hairy cell leukemia patients and improvement following interferon therapy. Cancer 1987 ; 60 : 2208-12.
62 Barak V, Nisman B, Polliack A. The tumor necrosis factor family and correlation with disease activity and response to treatment in hairy cell leukemia. Eur J Haematol 1999 ; 62 : 71-5.
63 Feldman M, Taylor P, Paleolog E, Brennan FM, Maini RN. Anti-TNF alpha therapy is useful in rheumatoid arthritis and Crohn's disease: analysis of the mechanism of action predicts utility in other diseases. Transplant Proc 1998 ; 30 : 4126-7.
64 van Dullemen HM, van Deventer SJ, Hommes DW, Bijl HA, Jansen J, Tytgat GN, et al. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). Gastroenterology 1995 ; 109 : 129-35.
65 Rutgeerts P, D'Haens G, Targan S, Vasiliauskas E, Hanauer SB, Present DH, et al. Efficacy and safety of retreatment with anti-tumor necrosis factor antibody (infliximab) to maintain remission in Crohn's disease. Gastroenterology 1999 ; 117 : 761-9.
66 Moreland LW. Inhibitors of tumor necrosis factor for rheumatoid arthritis. J Rheumatol 1999 ; 26 Suppl 57 : 7-15.
67 Baughman RP, Strohofer SA, Buchsbaum J, Lower EE. Release of tumor necrosis factor by alveolar macrophages of patients with sarcoidosis. J Lab Clin Med 1990 ; 115 : 36-42.
68 Raza A. Anti-TNF therapies in rheumatoid arthritis, Crohn's disease, sepsis, and myelodysplastic syndromes. Microsc Res Tech 2000 ; 50 : 229-35.
69 Bizzini B, Volpato I, Lachgar A, Cohen P, Gringeri A. IFN alpha kinoid vaccine in conjunction with tritherapy, a weapon to combat immunopathogenesis in AIDS. Biomed Pharmacother 1999 ; 53 : 87-9.
70 Bizzini B, Achour A. "Kinoids": the basis for anticytokine immunization and their use in HIV infection. Cell Mol Biol (Noisy-le-grand) 1995 ; 41 : 351-6.
71 Dalum I, Butler DMM, Jensen MR, Hindersson P, Steinaa L, Waterston AM, et al. Therapeutic antibodies elicited by immunization against TNF–alpha. Nat Biotechnol 1999 ; 17 : 666-9.