Available online at www.sciencedirect.com
SCIENCE DIRECT
Biomedicine & Pharmacotherapy 59 (2005) 474–480
BIOMEDICINE & PHARMACOTHERAPY
http://france.elsevier.com/direct/BIOPHA/

Dossier: HIV/AIDS: News approaches in chemotherapy and immunotherapy

Genomic approach of AIDS pathogenesis: exhaustive genotyping of the *TNFR1* gene in a French AIDS cohort

Gora Diop [a,b], Jean-Louis Spadoni [c], Hervé Do [a,b], Thomas Hirtzig [a,b], Cédric Coulonges [a], Taoufik Labib [a], Wolfgang Issing [d], Jay Rappaport [e], Amu Therwath [a], Mark Lathrop [b], Fumihiko Matsuda [b], Jean-François Zagury [a,c,*]

[a] *Équipe génomique, bioinformatique et pathologies du système immunitaire, Inserm EMI0355, 15, rue de l'École-de-Médecine, 75006 Paris, France*
[b] *Centre national de génotypage, 2, rue Gaston-Crémieux, 91057 Evry cedex, France*
[c] *Chaire de bioinformatique, conservatoire national des arts et métiers, 292, rue Saint-Martin, 75003 Paris, France*
[d] *Freeman Hospital, Newcastle upon Tyne NE7 7DN, UK*
[e] *Center for Neurovirology and Cancer Biology, Temple University, Philadelphia, PA, USA*

Received 26 July 2005

Available online 18 August 2005

Abstract

Large-scale genomic studies in cohorts have been made possible for the last few years thanks to the progress of molecular biology and bioinformatics. This systematic approach allows a better understanding of the molecular mechanisms of disease development and as a consequence can contribute to the rational design of new diagnostic and therapeutic tools. We present here the exhaustive genotyping of a candidate gene, tumor necrosis factor receptor 1 (*TNFR1*), in the genetic of resistance to immunodeficiency virus (GRIV) AIDS cohort. This gene was chosen because it is likely to be involved in the apoptosis pathways of $CD4^+$ and $CD8^+$ T-cells during human immunodeficiency virus 1 (HIV-1) infection. Seven frequent polymorphisms were characterized in 319 HIV-1 seropositive patients from the GRIV cohort with extreme disease progression phenotypes, slow progression or rapid progression, and in 427 healthy controls. The *TNFR1* gene locus does not appear to be part of any haploblock and contains only a small haploblock of two successive SNPs. One promoter SNP (TNFR1_17444594, position –581) and one intronic SNP (TNFR1_27223241, position +11511) gave weak positive signals of association (resp. $P = 0.03$ and $P = 0.04$) as well as two haplotypes. To our knowledge, this is the first genetic association study dealing with the *TNFR1* gene in AIDS and the putative associations identified will need to be validated through other AIDS cohort analyses or by further biological experimentation.

Keywords: AIDS; Gene; Polymorphism; Progression; Resistance; Tumor necrosis factor; TNFα

1. Introduction

The hallmark of HIV-1 infection is the progressive depletion of $CD4^+$ and $CD8^+$ T lymphocytes, which eventually leads to a major immunodeficiency corresponding to the AIDS stage of the infection [1]. The correlation between the depletion of T-cells in HIV-1 infection and apoptosis has been well-established by the study of the apoptotic pathways in large groups of patients [2,3]. Furthermore, observations performed on lymph nodes of infected patients have shown that apoptosis occurs predominantly in non-infected "bystander" cells, whereas cells productively infected with HIV-1 exhibit less apoptosis [4]. Several families of proteins are involved in the induction of apoptosis, such as FAS/FASL, tumor necrosis factor alpha (TNF-α), and tumor necrosis factor related apoptosis-inducing ligand (TRAIL) [5]. The particular role of the TNF-α/TNFR1 pathway in T-cell apoptosis has been thoroughly reviewed by Nagata [6]. The role of both FAS/FASL and TNF-α/TNFR1 pathways in the $CD4^+$ T-cells depletion during HIV-1 infection has been well documented [7–9]. An association between the susceptibility to TNF-induced apoptosis and the in vivo evolution of $CD4^+$ T-cell numbers has even been proposed [10]. Finally, the activation of the TNFR1 pathway by TNF-α or by specific antibodies triggers the expression of HIV-1 in infected cells [11,12]. All

* Corresponding author. Tel.: +33 1 58 80 88 20; fax: +33 1 53 10 93 03.
E-mail address: zagury@cnam.fr (J.-F. Zagury).

0753-3322/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.biopha.2005.07.011

these findings point out the significant role that the TNF-α/TNFR1 pathway may play in HIV-1 pathogenesis.

In this context, we have undertaken an extensive genotyping of the *TNFR1* gene in the 'genetics of resistance to immunodeficiency virus' (GRIV) cohort in order to determine if genetic variants could influence disease progression. The GRIV cohort consists of two sub-populations of Caucasian HIV-1 seropositive individuals living in France, with extreme progression phenotypes and gathers presently 100 patients with rapid progression (RP) and 300 patients with slow progression (SP). We also included 446 healthy control subjects of similar ethnic origin. The GRIV cohort corresponds to the extreme 1% subset of a cohort of 30 000 seroconverter patients [13] and it is, as far as we know, the largest cohort of its kind in the world. Its usefulness has already been validated for several gene polymorphisms such as CCR5 [14–16] and HLA [17,18]. Polymorphisms, mainly single nucleotide polymorphisms (SNPs), were identified through sequencing of the *TNFR1* gene and were evaluated for their association with disease susceptibility and progression.

2. Materials and methods

2.1. The GRIV cohort and control subjects

The GRIV cohort was established in 1995 in France to generate a large collection of DNA for genetic studies on candidate polymorphisms associated with rapid and slow progression to AIDS. Only Caucasians of European descent living in France were recruited. These criteria limit the influence of the virogenetic and environmental factors (subjects are all infected by B strains and live in a similar environment) and put emphasis on the genetic make-up of each individual to determine the various patterns of progression. Patients with slow progression (SP) were defined as seropositive asymptomatic individuals for 8 or more years with a $CD4^+$ cell count above 500 per mm^3 in the absence of antiretroviral therapy. Patients with rapid progression (RP) were defined as patients with a drop in their $CD4^+$ cell count below 300 per mm^3 in less than 3 years after the last seronegative test. The DNA was obtained from fresh peripheral blood mononuclear cells or from EBV-transformed cell lines. The control (CTR) subjects are seronegative Caucasians of European descent living in France.

2.2. Genotyping

The primers and conditions used for PCR amplification of the different fragments are presented in Table 1. Sequencing reactions were performed according to the Dye Terminator method using an ABI PRISM® 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Alignment, SNP discovery, and genotyping were performed with the software Genalys®, developed by the Centre National de Génotypage (CNG) [19]. For practical reasons, an initial screening was performed on 150 SP, 50 RP, and 150 CTR subjects for polymorphism discovery. The screening could then be extended on more subjects when a positive ($P \leq 0.05$) or borderline ($P \leq 0.1$) signal was detected. In this study, we have genotyped up to 238 slow progression patients, 81 rapid progression patients, and 427 control subjects.

2.3. Haplotypes

We estimated haplotypes using the EM algorithm [20] and the Phase2 algorithm [21,22] either for all polymorphisms or for selected ones.

2.4. Linkage disequilibrium and haploblocks

Linkage disequilibrium (LD) was computed for each pair of polymorphisms, using the r^2 [23] and the D' coefficients [24].

As defined by Gabriel et al. [25], a haploblock is a genetic region for which there is a low probability that a historical recombination event occurred: in other words, the SNPs located within that region exhibit a significant level of linkage disequilibrium. All identified polymorphisms with a minor allelic frequency above 1% have been tested for their belonging to a haplotype block (haploblock). The haploblocks in the genes have been computed using the method developed by Gabriel et al., which is utilized by the software Haploview [26].

2.5. Statistical analysis

Statistical analyses were performed only on the polymorphisms with a minor allele frequency greater than 1% in our whole population, termed 'frequent' polymorphisms. The differences in the allelic distributions (SNPs or haplotypes) between the three groups were examined as follows: for each

Table 1
Primers used to amplify the exons of *TNFR1* by PCR. The reaction mixture was: 5 μl DNA (5 ng/μl), 2.4 μl dNTP mix (2.5 mM each), 1.5 μl 10 × ExTaq™ buffer, 0.3 μl of each primer (10 μM), 0.15 μl ExTaq™ (Takara, Otsu, Shiga, Japan) (5 U/μl), and 5.35 μl water. Thermal cycling conditions for PCR were as follows: 94 °C, 10 min for one cycle; denaturation, 94 °C, 30 s; annealing, 55–60 °C, 30 s; extension 72 °C, 2 min for 40 cycles; and 72 °C, 10 min for one cycle

Exons	Forward primer	Reverse primer
1	5′-CAGAGACTGGATGAGACCTG-3′	5′-TCCAAAAGGCGGATGAATGG-3′
2–5	5′-GAATGTTTCACTGAGGAAGG-3′	5′-TCAGATTTGAAGAGCAAGGG-3′
6–8	5′-TAGCTGGCTACCTTCTCAAG-3′	5′-CAGGGACATTTGGGAGTAAC-3′
9–10	5′-TTCCCTCTAAGTCCCAACCC-3′	5′-ATCTCACCCCTCAGGATCTG-3′

allele (with a frequency greater than 1% in the whole population), the expected numbers of individuals in each group with and without that allele were compared using the Fisher's exact test. Four modes of calculation were used for the genetic analysis: allelic frequency, dominant mode, recessive mode, and genotypic distribution. For individual polymorphisms, there are only two alleles and the *P*-value for the dominant mode on one allele is identical to the *P*-value for the recessive mode on the other allele. As a consequence, Table 2 dealing with individual polymorphisms combines the dominant and recessive modes.

3. Results

3.1. SNP discovery

The *TNFR1* gene has been systematically screened for polymorphisms by sequencing exons with their flanking regions as well as the 1 kb-region upstream of the first exon, containing the putative promoter. Overall we identified 23 polymorphisms among which 7 have a minor allele frequency greater than 1% in the whole population (Fig. 1). As shown in Fig. 1, two SNPs are located in exons: TNFR1_17444602, located in exon 1, is synonymous (Pro12Pro, position taken from reference sequence NP_001056.1) and TNFR1_17444592, located in exon 4, introduces a non-synonymous change (Arg121Gln). TNFR1_17444593 and TNFR1_17444594 are located in the promoter region. Table 2 summarizes the frequency of each SNP in the SP, RP, and CTR populations, their association with AIDS progression and the relevant information known to date for each SNP. For all the SNPs, the allelic distributions fit with the Hardy–Weinberg expectations in the three SP, RP, and CTR populations ($P > 0.5$). The allelic frequencies obtained in our study are similar to the data provided by the NCBI dbSNP database regarding the European populations.

3.2. Gene structure

We investigated the presence of haploblocks in the *TNFR1* gene, i.e. genetic segments containing adjoining SNPs in linkage disequilibrium (LD) and thus presenting no evidence of historical recombination. Globally, the gene appears not to be part of a haploblock since many SNPs are not in LD (Fig. 2B,C). We could find only a small haploblock composed of the two successive SNPs TNFR1_17444599 and TNFR1_17536961 for which the confidence interval for D' is 0.96–1 (see Fig. 2C).

3.3. Associations with AIDS progression: SNPs

We performed statistical analysis to test whether the polymorphisms are associated with disease progression. By Fisher's exact test, two weak positive associations with AIDS progression were found for two SNPs (Table 2).

Table 2
List of all polymorphisms with a frequency greater than 1% in the global population for the *TNFR1* gene. For each polymorphism we indicated the allelic frequency in the different populations (CTR, RP, SP), the number of genotyped individuals, the *P*-values from the Fisher's exact tests, and the relevant information known to date. A1 represents the nucleotide which is identical to the reference sequence NT_009759.15. Column 1 shows the numbers attributed to the SNPs in the CNG database (CNG ID) and their position relative to the ATG start site, and column 2 gives their reference number in the db-SNP database. The calculation modes for the Fisher's exact test are indicated (AF: allelic frequency, D/R: dominant/recessive). Borderline (≤ 0.1) and significant (≤ 0.05) *P*-values are in bold. The *P*-values for the dominant/recessive calculation modes were only given when lower than 0.1. (*: Bonferroni corrections were not performed on *P*-values, NS: no significant values were detected)

SNPs		Reference	Alleles		A1 frequency (%)			Number of individuals			Nominal *P*-values for statistics test*						
											SP vs. CTR			RP vs. CTR		SP vs. RP	
CNG ID	Position/ +1ATG	to previous studies of the variant	A1	A2	CTR	RP	SP	CTR	RP	SP	AF	D/R	AF	D/R	AF	D/R	SP vs. RP vs. CTR AF
17444593	–609	rs4149570	T	G	39.11	42.60	36.34	427	81	238	0.346	NS	0.431	0.097	0.161	NS	0.332
17444594	–581	rs4149621, [28]	A	G	96.84	98.80	98.76	396	81	241	0.038	0.036	0.294	NS	1.000	NS	0.055
17444602	36	rs767455, [40]	A	G	58.29	53.30	59.18	199	46	158	0.819	NS	0.414	NS	0.338	NS	0.594
17444592	8335	rs4149584, [38]	G	A	96.83	98.00	95.49	142	50	144	0.515	NS	0.735	NS	0.372	NS	0.451
17444599	8635	rs1800692	T	C	35.14	43.40	38.01	138	53	146	0.487	NS	0.157	0.066	0.354	NS	0.326
17536961	10,972	rs1800693	A	G	59.80	65.70	58.74	153	54	143	0.802	NS	0.302	NS	0.248	0.0075	0.437
27223241	11,511	rs12426675	T	C	80.07	77.20	75.19	143	46	133	0.184	0.048	0.556	NS	0.779	NS	0.386

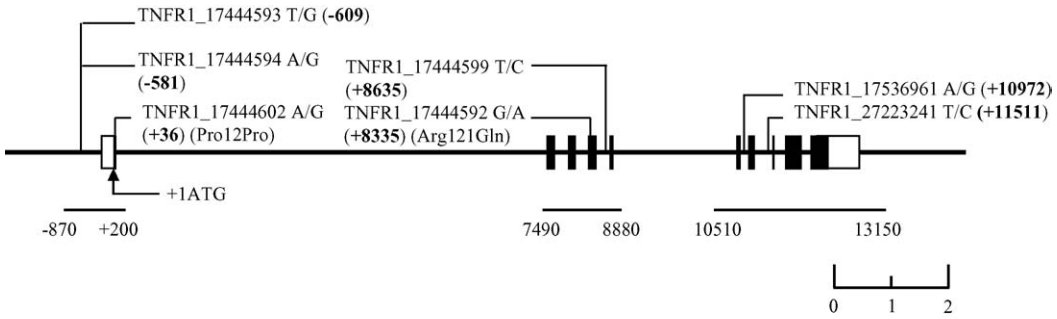


Fig. 1. Genetic organization of the *TNFR1* gene. Coding and untranslated regions are, respectively, indicated by solid and open rectangles. The regions that have been sequenced are indicated by a horizontal line, with starting and ending positions numbered in reference to the first nucleotide of the ATG codon, given as +1. The polymorphisms numbers are the attribution numbers from the CNG database (their position relative to the +1ATG is given in parenthesis, and the correspondence with Genbank SNP database is given in Table 2). The genomic sequence used for alignment is NT_009759.15.

TNFR1_17444594, located in the promoter region, exhibited *P*-values of $P = 0.036$ in dominant mode and $P = 0.038$ in allelic frequency mode when comparing the SP and CTR populations. TNFR1_27223241, located in intron 7, presented a *P*-value of 0.048 in the dominant mode when comparing the SP and CTR populations.

3.4. Associations with AIDS progression: haplotypes

We estimated the haplotypes using the Phase2 and EM algorithms, and identical results were obtained with both approaches. At first, we took into account all seven SNPs found in the gene. The computation yielded eight different haplotypes with a global frequency greater than 1%. Table 3 presents all the computed haplotypes, with their allelic frequencies, and *P*-values in the allelic frequency and the dominant mode (recessive mode yielded no significant association). Two of these eight haplotypes presented a positive signal (Table 3). The haplotype 2 presented significant associations for the comparison between the SP and RP populations ($P = 0.016$ in dominant mode) and for the comparison between the CTR and RP populations ($P = 0.024$ in dominant mode). The haplotype 5 presented significant associations for the comparison between the CTR and SP populations ($P = 0.038$ and $P = 0.034$ in allelic frequency and dominant mode, respectively). We also computed the haplotypes derived from the two SNPs located in the promoter region (TNFR1_17444593 and TNFR1_17444594), but there was no positive signal (data not shown). Finally, the sub-haplotypes derived from the haploblock made of the two SNPs TNFR1_17444599 and TNFR1_17536961 did not exhibit any association.

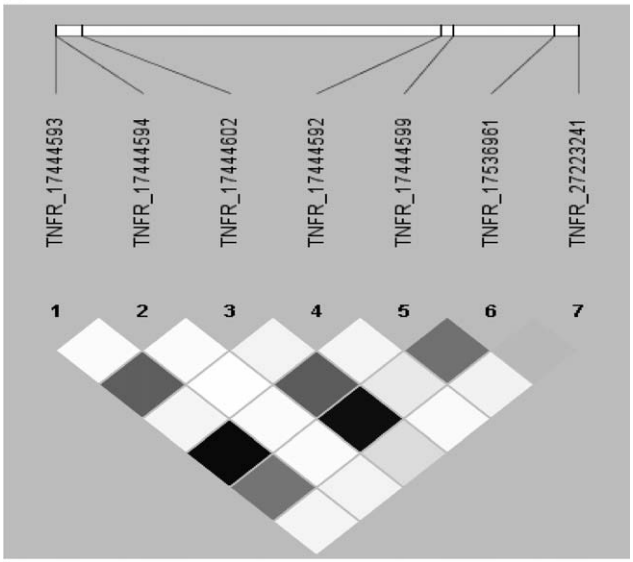
4. Discussion

We have undertaken the exhaustive genotyping of the *TNFR1* gene in order to identify new polymorphisms and possible associations with resistance/susceptibility to AIDS in the GRIV cohort. We found seven SNPs with a minor allele frequency greater than 1% in the whole population, but all of them had been previously described (Table 2). Only one small haploblock could be defined in the gene (Fig. 2). This latter observation is important to emphasize, because a trend is currently being developed: the systematic use of haploblocks to make genetic association studies. While this trend is legitimate when performing large genome-screenings, it appears insufficient when dealing with specific candidate genes. Indeed, it is also important to look for associations involving the sub-haplotypes derived from non-synonymous variations (i.e. mutations in the protein) or from promoter SNPs, for which a biological meaning may be easier to unravel.

Among the seven frequent SNPs, TNFR1_17444594 and TNFR1_27223241 exhibited positive signals ($P \leq 0.05$). TNFR1_17444594 is located in a 400-kb region upstream of exon 1 (located at –809/–385 relative to transcription start site) reported to have inhibiting properties [27]. The particular role of this SNP in gene transcription remains however unknown: competition in gel-shift assays or reporter gene experiments will have to be performed. The other SNP with a positive signal is intronic and no biological explanation can yet be provided for this putative association. There were also haplotypes exhibiting positive signals (Table 3). It appears difficult to provide a biological interpretation to these associations since these haplotypes are not linked to a single SNP allele.

To our knowledge, this is the first genetic association study on the role of *TNFR1* gene polymorphisms in AIDS. However, the *TNFR1* polymorphisms have been investigated in other diseases. In particular, the SNP TNFR1_17444602, corresponding to the synonymous mutation Pro12Pro, has been widely investigated by RFLP because the presence of its allele G creates an *Msp*A1 restriction site [28]. In contrast to our results in AIDS, associations have been described for this SNP in diseases such as Crohn's disease [29] or pancolitis [30]. Conflicting results have been found in Rheumatoid Arthritis: while the A/A genotype for this SNP has been shown to have a protective effect in a French population [31], it presented no association in other Caucasian populations [32–34]. No association for this SNP were found either in Alzheimer's disease [35] or in narcolepsy [36].

A



B

r^2	17444593	17444594	17444602	17444592	17444599	17536961
17444594	0.006					
17444602	0.474	0.006				
17444592	0.022	0.002	0.028			
17444599	**0.89**	0.007	0.48	0.022		
17536961	0.397	0.007	**0.845**	0.053	0.404	
27223241	0.021	0.028	0.091	0.009	0.035	0.19

C

D'	17444593	17444594	17444602	17444592	17444599	17536961
17444594	1 0.13-0.99					
17444602	**0.983** **0.91-1**	1 0.08-0.98				
17444592	1 0.43-1	0.077 0-0.43	1 0.25-1			
17444599	**0.953** **0.91-0.98**	1 0.13-0.99	**1** **0.95-1**	1 0.43-1		
17536961	**1** **0.96-1**	1 0.2-1	**1** **0.97-1**	1 0.67-1	**1** **0.96-1**	
27223241	0.347 0.12-0.54	1 0.39-1	0.62 0.41-0.76	1 0.14-0.99	0.445 0.22-0.62	**1** **0.92-1**

Fig. 2. A. Linkage disequilibrium map (r^2 coefficient) of the *TNFR1* gene provided by the software Haploview. If $r^2 > 0.5$ the LD is significant, and if $r^2 = 1$ it means that the LD is perfect.

B. Linkage disequilibrium table, based on the r^2 coefficient.

C. Linkage disequilibrium table, based on the D' coefficient.This coefficient is a normalized measure of the recombination occurring between two SNPs. The closer D' is to 1 the less recombination has occurred. The confidence intervals are also shown as D' can fluctuate easily if rare alleles or a small sample are involved.

TNFR1_17444592, the only SNP corresponding to a mutation in the protein, R121Q, exhibited similar allele frequencies in the three SP, RP, and CTR populations. The effect of the R121Q mutation on protein structure and function remains unknown. This SNP has been associated with an increased risk for atherosclerosis [37] or for a periodic-fever syndrome known as TNF receptor associated periodic syndrome (TRAPS) [38].

Overall we found some weak positive signals in our study suggesting that TNFR1 might be involved in AIDS disease development. We are aware that we did not perform any mul-

Table 3
Detailed information for the estimated haplotypes of the *TNFR1* gene. For each haplotype we indicated the global frequency, the frequency in each population SP, RP or CTR with the number of individuals in parenthesis, the *P*-value from the Fisher's exact test for the comparisons SP vs. CTR, RP vs. CTR, SP vs. RP and SP vs. RP vs. CTR, and the calculation mode. SP, RP and CTR represent the slow progression, rapid progression and control populations, respectively. The calculation modes AF and D are for the allelic frequency and the dominant modes, respectively. Borderline ($P \leq 0.1$) and significant ($P \leq 0.05$) *p*-values are in bold. (*: Bon ferroni corrections were not performed on nominal *P* values)

	Haplotype	1	2	3	4	5	6	7	8
SNPs	17444593	G	T	G	T	G	G	G	G
	17444594	A	A	A	A	A	A	A	A
	17444602	G	A	A	A	A	G	G	A
	17444592	G	G	G	G	G	A	G	G
	17444599	C	T	C	T	C	C	C	T
	17536961	G	A	A	A	A	G	A	A
	27223241	T	T	C	C	T	T	C	T
Allelic Frequency (%)	*CTR*	38.43 (103)	33.58 (90)	13.06 (35)	2.61 (7)	5.60 (15)	2.99 (8)	2.99 (8)	0.75 (2)
	RP	34.04 (32)	42.55 (40)	9.57 (9)	6.38 (6)	4.26 (4)	2.13 (2)	1.06 (1)	0.00 (0)
	SP	40.08 (105)	32.06 (84)	11.83 (31)	5.73 (15)	1.91 (5)	3.82 (10)	2.67 (7)	1.91 (5)
*Nominal p-values **	SP vs. RP	*AF* 0.325	0.077	0.704	0.801	0.251	0.739	0.686	0.331
		D 0.290	**0.016**	0.680	0.790	0.250	0.730	0.680	0.330
	RP vs. CTR	*AF* 0.460	0.130	0.460	0.110	0.790	1.000	0.460	1.000
		D 0.490	**0.020**	0.680	0.100	0.780	1.000	0.450	1.000
	SP vs. CTR	*AF* 0.720	0.713	0.695	0.083	**0.038**	0.639	1.000	0.281
		D 0.700	0.900	1.000	0.077	**0.034**	0.630	1.000	0.280
	SP vs. RP vs. CTR	*AF* 0.587	0.175	0.666	0.141	0.085	0.701	0.592	0.480

tiple tests corrections (Bonferroni corrections) in our computations: as previously suggested [13], our results will need to be confirmed or infirmed by other cohort studies in AIDS. Since the TNF-α/TNFR1 pathway appears to play a significant role in HIV-1 pathogenesis, this work will have also to be completed by the study of the *TNF-α* gene, which is the main ligand of TNFR1, and a known important factor in AIDS [39].

Acknowledgements

The authors are grateful to all the patients and the medical staff who have kindly collaborated on this project. They are thankful to A. Vasilescu for his critical help in performing the genotyping. This work was supported by ACV Development Foundation and Neovacs SA. The CNG is supported by the Ministere de la Recherche et des Nouvelles Technologies.

References

[1] Fauci AS. Host factors and the pathogenesis of HIV-induced disease. Nature 1996;384:529–34.
[2] Gougeon ML, Lecoeur H, Dulioust A, Enouf MG, Crouvoiser M, Goujard C, et al. Programmed cell death in peripheral lymphocytes from HIV-infected persons: increased susceptibility to apoptosis of CD4 and CD8 T cells correlates with lymphocyte activation and with disease progression. J Immunol 1996;156:3509–20.
[3] Holm GH, Gabuzda D. Distinct mechanisms of CD4+ and CD8+ T-cell activation and bystander apoptosis induced by human immunodeficiency virus type 1 virions. J Virol 2005;79:6299–311.
[4] Finkel TH, Tudor-Williams G, Banda NK, Cotton MF, Curiel T, Monks C, et al. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nat Med 1995;1:129–34.
[5] Krammer PH. CD95's deadly mission in the immune system. Nature 2000;407:789–95.
[6] Nagata S. Apoptosis by death factor. Cell 1997;88:355–65.
[7] Alderson MR, Tough TW, Davis-Smith T, Braddy S, Falk B, Schooley KA, et al. Fas ligand mediates activation-induced cell death in human T lymphocytes. J Exp Med 1995;181:71–7.
[8] de Oliveira Pinto LM, Garcia S, Lecoeur H, Rapp C, Gougeon ML. Increased sensitivity of T lymphocytes to tumor necrosis factor receptor 1 (TNFR1)- and TNFR2-mediated apoptosis in HIV infection: relation to expression of Bcl-2 and active caspase-8 and caspase-3. Blood 2002;99:1666–75.
[9] Alimonti JB, Ball TB, Fowke KR. Mechanisms of CD4+ T lymphocyte cell death in human immunodeficiency virus infection and AIDS. J Gen Virol 2003;84:1649–61.
[10] Aukrust P, Liabakk NB, Muller F, Lien E, Espevik T, Froland SS. Serum levels of tumor necrosis factor-alpha (TNF alpha) and soluble TNF receptors in human immunodeficiency virus type 1 infection—correlations to clinical, immunologic, and virologic parameters. J Infect Dis 1994;169:420–4.
[11] Edfjall C, Jacobsen H, Lotscher H, Mous J. Tumor necrosis factor receptor p55 mediates induction of HIV type 1 expression in chronically infected U1 cells. AIDS Res Hum Retroviruses 1996;12:199–204.
[12] Lazdins JK, Grell M, Walker MR, Woods-Cook K, Scheurich P, Pfizenmaier K. Membrane tumor necrosis factor (TNF) induced cooperative signaling of TNFR60 and TNFR80 favors induction of cell death rather than virus production in HIV-infected T cells. J Exp Med 1997;185:81–90.
[13] Huber C, Pons O, Hendel H, Haumont P, Jacquemin L, Tamim S, et al. Genomic studies in AIDS: problems and answers. Development of a statistical model integrating both longitudinal cohort studies and transversal observations of extreme cases. Biomed Pharmacother 2003;57:25–33.
[14] Rappaport J, Cho YY, Hendel H, Schwartz EJ, Schachter F, Zagury JF. 32 bp CCR-5 gene deletion and resistance to fast progression in HIV-1 infected heterozygotes. Lancet 1997;349:922–3.
[15] Hendel H, Henon N, Lebuanec H, Lachgar A, Poncelet H, Caillat-Zucman S, et al. Distinctive effects of CCR5, CCR2, and SDF1 genetic polymorphisms in AIDS progression. J Acquir Immune Defic Syndr Hum Retrovirol 1998;19:381–6.
[16] Winkler CA, Hendel H, Carrington M, Smith MW, Nelson GW, O'Brien SJ, et al. Dominant effects of CCR2–CCR5 haplotypes in HIV-1 disease progression. J Acquir Immune Defic Syndr 2004;37:1534–8.
[17] Hendel H, Caillat-Zucman S, Lebuanec H, Carrington M, O'Brien S, Andrieu JM, et al. New class I and II HLA alleles strongly associated with opposite patterns of progression to AIDS. J Immunol 1999;162:6942–6.
[18] Flores-Villanueva PO, Hendel H, Caillat-Zucman S, Rappaport J, Burgos-Tiburcio A, Bertin-Maghit S, et al. Associations of MHC ancestral haplotypes with resistance/susceptibility to AIDS disease development. J Immunol 2003;170:1925–9.
[19] Takahashi M, Matsuda F, Margetic N, Lathrop M. Automated identification of single nucleotide polymorphisms from sequencing data. J of Bioinformatics and Computational Biology 2003;1:253–65.
[20] Laird N. The EM algorithm. In: Rao C, editor. Handbook of statistics vol. 9: computational statistics. Amsterdam: Elsevier Science Publishers B.V.; 1993. p. 509–20.
[21] Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–89.
[22] Stephens M, Donnelly P. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003;73:1162–9.
[23] Hill WG, Weir BS. Maximum-likelihood estimation of gene location by linkage disequilibrium. Am J Hum Genet 1994;54:705–14.
[24] Devlin B, Risch N. A comparison of linkage disequilibrium measures for fine-scale mapping. Genomics 1995;29:311–22.
[25] Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. Science 2002;296:2225–9.
[26] Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–5.
[27] Kemper O, Wallach D. Cloning and partial characterization of the promoter for the human p55 tumor necrosis factor (TNF) receptor. Gene 1993;134:209–16.
[28] Pitts SA, Olomolaiye OO, Elson CJ, Westacott CI, Bidwell JL. An MspA1 I polymorphism in exon 1 of the human TNF receptor type I (p55) gene. Eur J Immunogenet 1998;25:269–70.
[29] Waschke KA, Villani AC, Vermeire S, Dufresne L, Chen TC, Bitton A, et al. Tumor necrosis factor receptor gene polymorphisms in Crohn's disease: association with clinical phenotypes. Am J Gastroenterol 2005;100:1126–33.
[30] Pierik M, Vermeire S, Steen KV, Joossens S, Claessens G, Vlietinck R, et al. Tumour necrosis factor-alpha receptor 1 and 2 polymorphisms in inflammatory bowel disease and their association with response to infliximab. Aliment Pharmacol Ther 2004;20:303–10.
[31] Dieude P, Osorio J, Petit-Teixeira E, Moreno S, Garnier S, Cailleau-Moindrault S, et al. A TNFR1 genotype with a protective role in familial rheumatoid arthritis. Arthritis Rheum 2004;50:413–9.
[32] Barton A, John S, Ollier WE, Silman A, Worthington J. Association between rheumatoid arthritis and polymorphism of tumor necrosis factor receptor II, but not tumor necrosis factor receptor I, in Caucasians. Arthritis Rheum 2001;44:61–5.

[33] Bayley JP, Bakker AM, Kaijzel EL, Huizinga TW, Verweij CL. Association of polymorphisms of the tumour necrosis factor receptors I and II and rheumatoid arthritis. Rheumatology (Oxford) 2003;42:969–71.
[34] Glossop JR, Nixon NB, Dawes PT, Hassell AB, Mattey DL. No association of polymorphisms in the tumor necrosis factor receptor I and receptor II genes with disease severity in rheumatoid arthritis. J Rheumatol 2003;30:1406–9.
[35] Perry RT, Collins JS, Wiener H, Acton R, Go RC. The role of TNF and its receptors in Alzheimer's disease. Neurobiol Aging 2001;22:873–83.
[36] Wieczorek S, Dahmen N, Jagiello P, Epplen JT, Gencik M. Polymorphisms of the tumor necrosis factor receptors: no association with narcolepsy in German patients. J Mol Med 2003;81:87–90.
[37] Poirier O, Nicaud V, Gariepy J, Courbon D, Elbaz A, Morrison C, et al. Polymorphism R92Q of the tumour necrosis factor receptor 1 gene is associated with myocardial infarction and carotid intima–media thickness—the ECTIM, AXA, EVA and GENIC Studies. Eur J Hum Genet 2004;12:213–9.
[38] Aksentijevich I, Galon J, Soares M, Mansfield E, Hull K, Oh HH, et al. The tumor-necrosis-factor receptor-associated periodic syndrome: new mutations in TNFRSF1A, ancestral origins, genotype–phenotype studies, and evidence for further genetic heterogeneity of periodic fevers. Am J Hum Genet 2001;69:301–14.
[39] Gougeon ML. Apoptosis as an HIV strategy to escape immune attack. Nat Rev Immunol 2003;3:392–404.