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Contribution of cohort studies in understanding HIV pathogenesis: introduction of the GRIV cohort and preliminary results

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Summary – In the present paper we review studies performed on HIV-infected patients cohorts in order to understand AIDS disease development. The interplay between diverse factors such as the HIV envelope proteins, cellular co-receptors, the immune response with chemokines and cytokines production define the viral tropism, cytopathicity and progression of HIV disease. We present the trends of the research particularly in the domain concerning host genetics. In this context, we describe the GRIV cohort of fast and slow/non-progressors, and its use for understanding basic features of the yet unknown HIV pathogenesis mechanisms.

INTRODUCTION

The course of HIV-infection may depend on host genetics, viral genetics or environmental factors such as co-infections. Initial studies on cohorts of patients have been very useful for understanding mainly the immunological and virological factors involved in disease progression [1–6]. Since 1994, reports on these cohorts have put forth new concepts regarding disease evolution such as: slow or non-progression [1–4], fast progression [5, 6], low CD4 long term survival [7] and the existence of multiply HIV-exposed uninfected subjects [8, 9]. The concept of "non-progressor" was first introduced in the report of the San Francisco City Cohort, where gay men infected since 1978 (over 15 years) were observed to be still in good health with well-maintained biological parameters [10]. The concept of "fast progressor" was developed in parallel, illustrating the opposite extremes of clinical evolution. Since then, numerous biological studies have dealt with such cohorts primarily on phenotypical parameters such as the immune status, or on virological parameters. With the progress of human genetics, it is possible to also study the host genetics. Results concerning comparisons of HLA or CKR5 chemokine receptor have been generated. Cohorts specifically dedicated to host genetics such as the GRIV cohort established by our group are now being gathered.

In the present paper, our goal is to briefly review the major results obtained from cohort studies, and the trends of the research in the area of HIV molecular immunology and to introduce the GRIV cohort.

VIROLOGICAL AND IMMUNOLOGICAL FACTORS

Virological factors

The major viral phenotype associated with HIV-virulence has been attributed to syncitum-inducing (SI) strains: in fact, it has been possible to culture SI strains most often from patients with AIDS while infected asymptomatic patients rarely exhibited SI viruses. A shift from non SI (NSI) to SI strain is a predictive marker for progression towards AIDS [11–16]. In general, SI strains are T-lymphotropic while NSI are rather macrophage-tropic, dual-tropic infecting both T-cells and macrophages. With the recent works published on chemokines and their receptors, it now clearly appears that NSI/SI phenotypes are associated with

tropism for C-C chemokine receptors such as CKR5 (NSI strains) or the fusin receptor (SI strains). Cell tropism, SI/NSI phenotype, and receptor choice appear to be governed by sequences within the V3 loop of HIV envelope glycoprotein [8, 17]. This notion is attractive since a shift from NSI to SI strain would correspond to a slow genetic drift in the V3 loop sequences, probably induced by the selective pressure of chemokines [18] and/or the immune response [19].

Following the paper published by Daniel et al where a *nef*-deleted virus was shown to be non-pathogenic [20], non-progressors have been the object of intense scrutiny for *nef* deletions. Such deletions have been found only in a small number of non-progressor individuals, and more importantly in a cluster of blood recipients infected from a single source [21, 22]. These data give strong support for the use of *nef*-deleted viruses as vaccines. However, recent evidence from studies on SIV-(Simian) infected new-born macaques have emphasized the potential risk of such vaccines [23].

Immunological factors

HIV infection follows a pattern of evolution which could schematically be summarized as follows [24]:

— a primary infection with significant viral load;
— a period of clinical latency with low viral burden but progressive decline in CD4 cells and chronic immune activation;
— a phase with faster decline of CD4 cells and decline of CD8 cells probably with higher viral load;
— and AIDS characterized by profound immunosuppression and highly replicating virus.

The viral load has been studied on patients cohorts and allowed to define this "clinical latency" period with relatively low viral burden, when the increase in viral load was associated to disease progression and poor prognosis, in particular for the virus load early in infection [4, 25–29]. However, during the "clinical latency phase", the low virus burden suggests that the viral replication and colonisation is under negative control by the immune system. It is the collapse of immune functions which thus would allow for increased virus replication at late stage of disease. The increase in viral load may result from destruction of follicular dendritic cells (FDC) which trap large quantities of viral particles at the cell surface [24]. It is conceivable that SI strains are more detrimental to FDC and the ultimate involution of the lymph node may be responsible for the observed increase in circulating virus. Thus the failure to trap circulating virus may be contributing to increased viral loads late in the disease. Interestingly, the shift from NSI to SI strains seems to precede the increase in viral load [30]. A model of viral pathogenesis might then be multiphasic involving stages of immune dysfunction, viral dissemination, replication, evolution and finally lymphoid destruction taking place in parallel at different loci in the body.

Two major publications promote the previous view of disease evolution: a first publication on the Amsterdam cohort in 1992 which suggests a biphasic rate of CD4 decline [16] and a publication on the MACS cohort (Multi AIDS Cohort Study) in 1995 [31]. This latter publication shows that the total lymphocytosis is a homeostatic parameter in humans which is conserved in HIV-infected people until up to 18 months (on average) before the apparition of AIDS clinical events: the decline of CD4 cells is compensated by an increase of the number of CD8 cells. In fact, many papers have emphasized on the appearance of activation markers such as DR and CD38 on CD4 and CD8 cells [32, 33] or CD28 on CD8 cells [34, 35] which promotes the idea that CD8 cells are actively proliferating in the course of infection. Also, a cross-sectional study on a small cohort has shown a decrease in CD8 cells telomeres size, marker of cell proliferation, which does not apparently occur for CD4 cells [36].

Other studies have emphasized the role of shifting cytokines production pattern [37] in HIV infected patients, but this latter result has not been studied extensively on cohorts up to now probably since it implies heavy cell culture operations.

For many other immunological parameters rapid and non-progressors can be compared: well-preserved humoral and cell-mediated immune response in SP while strong diminution of these responses in RPs [2]. For instance, low level of neutralizing antibodies directed towards autologous variants in RPs while high level in SPs, absence of anti-HIV CTL activity in RPs while strong response in SPs [2]. In the same manner, high level of serum markers for immune activation in RP versus low level in SP [2].

The cohort ALT [38] was designed initially to identify phenotypical parameters and viral para-

meters in non progressor patients. The inclusion criteria chosen for non progressors were basically: subjects asymptomatic with stable CD4 cell counts, higher than 600 after 8 years of infection. After one year of study of 50 such patients, about one-third of them underwent a drop of their CD4 cells under 600. In these patients the viral load increased significantly, the anti-HIV cytotoxic T-lymphocyte (CTL) activity decreased including that against regulatory genes such as *tat*. Vβ repertoire modifications were also found [38].

The average production of chemokines as well as other cytokines such as interferon-α (IFNα) in highly exposed uninfected subjects is higher than in the normal population. This has been shown in a study by our group on a cohort uninfected HIV-exposed hemophiliacs (D Zagury et al, submitted for publication). Moreover, none of these subjects were homozygous for the CKR5 deletion identified in some subjects resistant to HIV infection (see below). Such level of production is probably genetically controlled and deserves extensive research.

HOST GENETICS FACTORS

Numerous studies have been performed in order to explain the role of the host genetic background in determining the rate of the progression of the disease.

HLA: class 1, class II, and TAP genes

There has been considerable interest in searching for an HLA association with HIV disease progression. The HLA haplotype of an individual should play a major role in determining disease susceptibility and clinical outcome. All studies rely on computations of the relative risk (RR) associated with the presence of specific alleles or combination of alleles in candidate genes in the study population compared to a control population. We have looked only for highly significant relative risks (RR over 3) since, due to small numbers of subjects in the studies and unknown alleles associations (linkage imbalance), relative risks of lesser power might be unreliable.

The Multi AIDS Center Cohort Study (MACS) has been established since 1984 [39] and has included over 400 patients followed since their seroconversion, while the DC gay cohort (DCG) has been created since 1982 and included people already infected in 1982 [40].

In a study published recently by Kaslow et al [41], two comparable groups of patients were taken from each cohort: caucasian patients, 71 with AIDS and 68 AIDS-free in MACS while 66 with AIDS and 36 AIDS-free in DCG. Predictive association HLA markers for disease evolution were computed from the first cohort (MACS). These newly defined association markers were then applied on the second cohort to confirm their validity. There was an overall agreement on the prognostic parameters defined by the MACS HLA markers when applied on the DCG population. To summarize, markers such as B27, B57, A25 & TAP2.3 were associated with slower progression while A28 & TAP2.3, and A23 & not TAP2.3 were associated with faster progression with significant relative risk (over 3).

The study published by Kaslow is strengthened by the consistency of results between two independent cohorts. Many other additional studies had been performed by other investigators using a variety of genetic markers. An interesting study was published in 1990 by Kaplan et al concerning a cohort of patients followed by the INTS in Paris [42]. The authors report specific associations with B8, B8 & DR3, BW21 for rapid progression, while A11 and DR4 are associated to slower progression with a significant relative risk (over 3). This study confirms a former study by Steel et al [43] on hemophiliacs from Edinburgh which showed a higher risk of infection and a faster evolution on people with the A1 & B8 & DR3 (often inherited en bloc) haplotype. These results are in agreement with those obtained on a cohort of 243 HIV-infected born children who had a poor prognosis if they had the allele DR3 [44]. They are also in agreement with those obtained on a cohort of hemophiliacs in Italy which show that DR3 (RR 12) and DQw2 (RR 25) are highly associated with disease progression [45]. Another study published by Kaslow in 1990 [46], on patients from the MACS cohort, showed that A24 was associated with fast progression, that A1, Cw7 and B8 were also slightly associated with progression and any combination of the three was significantly associated with progression. Some groups explain the effect of the A1&B8 HLA haplotype by its association with a C4 complement gene deletion – C4 null –, which could impair their immune defense: this work has been done on an Australian cohort [47], and on a cohort of patients in Luxembourg [48]. Other studies have dealt

with HLA-B35 and fast progression [49–54]: only in some of these studies, the relative risk for fast progression is above 3 [52–54]. However the risk seemed to change according to the population studied (hemophiliacs, intravenous drug users, homosexuals) [54]. Another interesting study was done on a cohort of patients from Mississipi where they distinguished the HLA parameters of African Americans (Aframs, 38 individuals) and caucasians (Caucas, 24 individuals) subpopulations for fast or slow progression [55]. Among Caucas the DQ3 and the haplotype A30(19)-B67 were associated with slow progression, while DQ2 and haplotype A28-B17-DR9 were associated with fast progression. Among Aframs the DQ1 was associated with slow progression, while haplotypes A69(28)-B40 and B12-DR14 were associated with fast progression. However the interpretation of these results is limited by the small number of subjects.

Many other studies were published presenting associations between HLA an fast/slow progression: we do not mention them because they did not reach our cut-off of 3 for the relative risk, which is a reliable threshold for significant associations. In fact, Kaslow and Mann have underlined very clearly the possible caveats of making HLA locus associations with disease and explain the discrepant results obtained by different groups according to the population they studied (even though from same ethnic background) the criterias they chose as well as the technology they used [56].

In the previous paragraphs we described some data obtained about HLA and rate of disease progression: it has now become more and more interesting to study the multiple exposure uninfected subjects. For instance, resistance to HIV infection of women prostitutes in Nairobi has been associated with HLA-A28 and HLA-DR13 [57]. The effect of HLA alleles, however, may depend on other genetic factors which may vary among cohorts. Cruse et al [58] compared the prevalence of HLA alleles in their cohorts Caucas and Aframs in comparison to control caucasian and African American populations from the same region, and they found that, among Aframs A28 was not associated with resistance or susceptibility to infection. However, for Caucas the presence of A28 corresponded to an increased susceptibility to HIV-infection with a RR of 4! The apparent contradiction between these data and those obtained on Gambian prostitutes could be a clue to favored allelic associations in HLA haplotypes which may differ from one population to the other. Effects of multiallelic association may be difficult to interpret. In the same publication concerning the Mississipi cohort, the authors show however some alleles associated to increased susceptibility to HIV infection with very high RR in both Caucas and Aframs: these alleles are Bw70, Cw6, Cw7, DRw12, DQw6, DQw7. Finally in Milan, Fabio et al also found on a cohort of 31 highly exposed uninfected hemophiliacs that the A2 allele was significantly associated with non infection compared to 31 seropositive hemophiliacs [45].

Some HLA markers have been associated with certain clinical manifestations such as HLA-DR1 with Kaposi sarcoma while rather preventing occurrence of opportunistic infections [53, 59], or B62 with fever and skin rash, as found in a seroconverters cohort in Amsterdam [53].

Chemokines and their receptors

Since the seminal publication by Cocchi et al [60] on the inhibitory effect of chemokines on HIV replication it has been demonstrated that HIV macrophage-tropic strains use chemokine receptor CKR5 as a co-receptor for infection [17, 18, 61–63], while HIV T-lymphotropic strains use rather a new member of the chemokine receptor family termed fusin [64]. Two recent publications have shown that a 32 bp deletion in the CKR5 receptor found in certain caucasian subjects, leading to the expression of a truncated receptor, is associated to resistance to HIV infection by these subjects [65, 66]. These two publications have been completed by an extensive study by Dean et al [67] on a pool of six cohorts of individuals from high risk groups followed in the US: HGDS (Hemophilia Growth and Development Study) [68], MHCS (Multicenter Hemophilia Cohort Study) [69], DCG (homosexual men and intravenous drug users from Washington DC) [39], MACS (homosexual men from New York) [40], SFCC (homosexual men from San Francisco) [10], ALIVE (AIDS Link to the Intravenous Experience) [70]. These six cohorts totalized 1, 955 individuals, 612 being uninfected. The results of the study clearly showed that the allelic frequency of the CKR5 deletion is 10%, that homozygous people for the CKR5 deletion are ap-

parently resistant to infection by HIV-1, and that heterozygous people are statistically progressing more slowly. Heterozygous people are in the same proportion among infected and uninfected populations which means that heterozygosity does not seem to prevent infection.

The recent identification of fusin as the co-receptor for T-lymphotropic (or SI) strains should also lead to genetic studies on this receptor. Mutations in this receptor might prevent disease evolution in non-progressors by inhibiting infection by SI strains. Of course, the polymorphism of the chemokines ligands of CKR5 (RANTES, MIP1α and β) or of the C-X-C chemokine SDF-1 which is the first known ligand of fusin [71, 72], should also to be studied.

THE GRIV COHORT

The GRIV cohort is a cohort of both slow/non-progressors and fast progressors. The criteria of inclusion for non progression was defined as follows: CD4 cell count above 500/mm^3, asymptomatic since primary infection, no antiretroviral treatment except for a short period (case of pregnant women and thrombocytopenic patients), subject of caucasian origin (caucasian grand-parents born in Europe). The criteria of inclusion for fast progression was defined as follows: CD4 cell count having dropped below 300 less than 3 years following seroconversion (this includes the symptomatic primo-infections with CD4 cells drop), subject of caucasian origin (caucasian grand-parents born in Europe).

The objective of the cohort is to determine genetic polymorphism associated with pertinent candidate genes, compared to a control population in order to identify some clues in the mechanisms of HIV-1 pathogenesis. The advantage of our cohort is that fast progressors can serve as a mirror control to non-progressors.

The collection of samples is done throughout France with the collaborative participation of many nurses, physicians and hospitals. The project started in September 1995, and about 200 samples were collected within a year: 150 slow/non progressors, 50 fast progressors. GRIV cohort is the largest homogeneous cohort of slow/non progressors gathered to our knowledge. Our goal is to collect more than 400 samples over the next two years. GRIV cohort recruits subjects at the two extremes of the gaussian curve of the time for CD4 cell decline after infection: according to our experience with the criteria chosen, our cohort corresponded for each group (fast and slow/non progressors) to about 1-2% of the people followed in the collaborative centers. This means that the 200 samples gathered up to now correspond to the survey of a cohort of about 10,000 patients.

Tables I and II present some characteristics on the GRIV cohort. Unfortunately, all the case report forms have not been received and the table concerns only 113 of the subjects already enrolled. Among these 113 cases, 78 were non progressors, and 35 fast progressors. Among the slow/non progressors, 59 were males (76%), 19 females (24%) while among the fast progressors 27 were males (77%) and 8 females (23%). The average age of the subjects enrolled is about 35 years in all subgroups. The fast progressors cases often corresponded to recent infections and it is logical that we did not get samples from people infected by blood transfusion (most often hemophiliacs). More observations will be drawn from the total collection of the case report forms.

Some studies have already been performed on the cohort samples: comparison of viral load, serum interferon levels, anti-p24 antibodies as predictive markers for disease progression (YY

Table I. Slow/non-progressors.

Mode of contamination	*Male*	*Female*	*Age/group (mean, median, SD)*
Homosexual	31	0	*38, 36, 8*
Heterosexual	3	9	*33, 33, 5*
Intravenous drug user	21	8	*35, 34, 5*
Blood transfusion	3	2	*36, 38, 11*
Other	1	0	*57*
Age/sex (mean, median, SD)	*37, 35, 7*	*34, 33, 5*	

Table II. Fast progressors.

Mode of contamination	*Male*	*Female*	*Age/group (mean, median, SD)*
Homosexual	17	0	*35, 33, 10*
Heterosexual	8	7	*30, 28, 8*
Intravenous drug user	1	1	*36, 36, 8*
Blood transfusion	0	0	–
Other	1	0	
Age/sex (mean, median, SD)	*33, 20, 39*	32, 34, 7	39

Cho, submitted for publication), size of telomeres as markers of cell replication (J Rappaport, in preparation), CKR5 deletion analysis as a marker for non-progression (H Hendel, in preparation). Besides the genes already mentioned in this article, the candidate genes studied will be those involved in immune regulation such as cytokines and their receptors, cluster differenciation markers, etc.

CONCLUSION

In this review we have presented major results concerning HIV immunopathology discovered through the study of patients cohorts. These results concern particularly the appearance of HIV SI strains which seem to precede the onset of clinical manifestations for about 2 years [16], as well as the rupture of lymphocytosis homeostasis [31] which also precedes the onset of clinical events for about 18 months in average. The link between the appearance of SI strains and the failure of T-cell homeostasis should be investigated.

Many studies have initially dealt with host genetics of HLA, even though often difficult to interpret [56], and with the recent discovery of the role of chemokines a major focus has already been placed on CKR5, and probably soon on other chemokine receptors. The GRIV cohort should enable us to make inroads into the pathophysiology of AIDS, to identify relevant genetic markers and finally lead to the development of novel therapeutic agents.

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