CHAPTER V

DISCUSSION

The patients under this longitudinal natural history study were recruited during 1986 to 1990, the time when HIV infection first arrived in Thailand. Male homosexuals and bisexuals were the victims of the first wave of HIV epidemic in Thailand, therefore, the majority (52.5%) of the patients recruited into the study were male homo/ bisexuals (Table V). It also explains why two-third of the symptomatic HIV patients (ARC and AIDS) at entry into the study were male homo/bisexuals since they had been infected longer. It was interesting to note that there were more male heterosexual patients than IVDU patients (Table V) even though IVDUs as a group, were infected earlier than the general male heterosexual population. The reason may be because most of the IVDU, unlike the male heterosexuals, did not complete the 2-year follow-up.

It can be derived from our preliminary natural history study that 13.5% (7 out of 52) of the asymptomatic HIV patients including PGL will progress to symptomatic HIV stages (ARC, AIDS, death) in 2 years, (Table VI), an annual rate of 6.8% of disease progression. This is similar to the rate of disease progression reported in Western countries of 30% per 5 years or 6% per year[108]. It also became obvious in our study that all ARC patients would progress within 2 years. And, once AIDS is diagnosed, one-third will die in 2 years (Table VI).

It was obvious from our study that HIV patients as a group, would have some abnormalities of their T lymphocyte

phenotypes as early as on the first diagnosis of their infections (Table VII). Most of the HIV infections in our patients started no longer than 2 years prior to the diagnosis (unpublished observations). Many had negative anti-HIV tests 3-6 months earlier. The changes were most obvious with the percentage and the absolute number of CD_4^+ T cells and the CD_4/CD_8 ratio, but were less obvious with total T cell count as well as the percentage and the absolute number of CD_8^+ T cells (Table VII). β_2-microglobulin levels were also significantly elevated in all groups of HIV patients at diagnosis. As compared to the normal controls, the reduction in the percentage and the number of CD_4^+ T cells, the reduction in the absolute number of CD_8^+ T cells, the reduction in CD_4/CD_8 ratio, the reduction in the number of total T cells and the increase in the level of β_2-microglobulin were most marked in AIDS, followed sequentially by ARC and asymptomatic HIV infection (Table VII). The values in PGL were almost identical to those of the asymptomatic HIV infection indicating that these 2 groups are immunologically equivalent or may reversibly interchange. The differential immunologic dysfunctions with advancing HIV infection observed in our study confirm the findings of other studies in the Western hemisphere[18,22,88].

When the above mentioned immunologic parameters were reanalysed at 24 months (M24), it was evident that there were universal reductions of mean total T cells, CD_4^+ percentage, CD_4^+ number, CD_8^+ number and the increase of β_2-microglobulin levels (Table VII). The annual decline of mean CD_4^+ number was surprisingly constant for different stages of HIV infection (Table XIII). A mean decrease of 20% of the initial values of CD_4^+ number was observed per year (Table XIII). This rate of drop of CD_4^+ number was consistent with the observation in other

studies[18,88]. If this rate of decline in CD_4^+ cells holds true for a larger patient population being followed for a longer period, it will be very useful in predicting disease progression and in determining the appropriate time to start antiviral or immunopotentiating drugs.

Table XIII : Calculated Annual Reduction of CD_4^+ T Cell Numbers
in Patients with Various Stages of HIV Infection

Stage of HIV Infection at Entry	N	Mean Number of CD_4^+ T cells		Percent Decrease per Year
		Month O	Month 24 or Near Death	
AIDS	6	318	162	24.5 %
ARC	3	515	337	17.3 %
PGL	34	1,049	629	20.0 %
Asymptomatic	18	964	609	18.4 %

Mean Annual Decrease = 20.1 ± 3.2 %

Out of the 61 patients who have been followed for at least 2 years, 16 progressors and 45 non-progressors could be defined at the end of 2 years. They were not significantly different in terms of age, sex or risk behaviors (Table VIII). However, progressors and non-progressors are clinically defined and arbitrarily divided. That is number of progressors after 1 year for instance, will certainly be less than that established after 2 years or 3 years. Therefore, it will be of great interest and importance if certain immunologic parameters can be identified at first evaluation of patient which can correctly predict that whose disease is going to progress. Several means of approach to answer this question were made in our study.

If the unstratified values of all immunologic markers were analysed (Table IX) only the initial (MO) CD_4^+/CD_8^+ ratio and the initial (MO) β_2-microglobulin levels of the progressors and non-progressors were significantly different. The percentage and number of CD_4^+ cells will become discriminatory only after having had the infection for at least 1 year if one assumes that most of our patients were newly infected by the time that they came to our clinic as discussed earlier.

The absolute number of CD_8^+ cells and the total T cell number of the progressors also started to become significantly less than the non-progressor group from 12 months on (Table IX). Therefore, immunologic re-evaluation of HIV patients 12 months after known seropositivity is essential in patient follow-up. The changes in the laboratory values at 12 months may be important markers for disease progression in that patient. Figure 23 (page 76) illustrates the changes in CD_4^+ number in 2 patients from the time of known seroconversion to the development of full-blown AIDS. It is also of interest to point out from Table IX that the β_2-microglobulin levels of the non-progressor group increased gradually with time suggesting that there is progressive lymphocyte activation in HIV infection as previously described[29]. However, such increase in B_2-microglobulin does not correlate with disease progression but with length of HIV infection.

To determine whether the cellular (CD_4^+, CD_8^+) or serologic markers (β_2-microglobulin, p24 antigen, anti-p24) can be used to predict the course of HIV infection and the development of AIDS, prospective evaluation of these immunologic parameters was carried out in our study. It was found that these markers had different degrees of usefulness in predicting disease progression. All measurements of CD_4^+ T cells had strong

correlation with subsequent progression to AIDS. The low CD_4^+ T cells, the elevated β_2-microglobulin level and the disappearance or reduction of serum anti-p24 concomitant with the appearance of p24 antigen can be reliably used as predictors for AIDS progression in the present study. These findings are consistent with other previous reports[25,26,27,28]. In fact, the relative hazards reported in our study were comparable to those of Fahey et al[88] although the number of patients in both studies was different. For example, in our study, in the group of pateints with CD_4 less than 20% (N=10), the relative risk of developing AIDS was 5.8 whereas that of Fahey et al was 7.8 for patients with CD_4 less than 18% (N=25). However, the percentage of CD_8^+ T cells and CD_4/CD_8 ratio had no association with subsequent progression to AIDS (Table X). In this aspect, our findings are different from those reported by Fahey JL et al [88] and Bhalla RB et al [19] which indicated that CD_4/CD_8 ratio and β_2-microglobulin levels were reliable markers for HIV progression respectively. The discrepancy may be due to different patient populations, different stratification of the markers or the number of patients in each stratification.

When correlations among different markers were analysed, it was found that different cellular markers (namely CD_4^+ percentge, CD_4^+ number, CD_8^+ number and CD_4/CD_8 ratio) were intercorrelated (Table XI). This was also true for serologic markers (i.e., β_2-microglobulin and p24 antigen levels). Therefore, for the sake of convenience and economics, one lymphocyte marker (preferrably, CD_4^+ number) and one serologic marker (preferrably p24 antigen level) may be selected as routine laboratory prognostic markers for HIV patients. The question is then how reliable are the combined markers in predicting HIV progression.

By combining serologic markers with the measurements of CD_4^+ T cells, it was found that only the p24 antigen levels in combination with the percentage or the absolute number of CD_4^+ T cells was more useful in predicting prognosis than either was used alone. For example, the relative risks for CD_4 <20% and for positive p24 antigen alone were 5.8 and 5.9 respectively (Table X), but for the combination was 30.3 (Table XII). These findings indicate the importance of both immune deficits (decreasing CD_4^+ T cells) and viral reactivation (increasing p24 antigen) as a result of immune stimulation in predicting prognosis. Mitogen-induced cytokine production and cellular proliferation were associated with increased production of HIV[109]. However, another marker of immune stimulation, namely, β_2-microglobulin, was not found to increase the prognostic value of CD_4^+ T cells in our study (Table XII). This was contrary to the findings of Fahey et al[88]. The difference may be due to the smaller number of patients in our study.

Although HIV antigenemia associates well with the progression of the disease[110,111], p24 antigen is hardly detected in serum of HIV- infected patients even with full-blown AIDS[112]. In the present study, it was shown that p24 antigen could be reliably used as prognostic markers with relative hazard of 5.9 (Table X).

Combining CD_4^+ T cells with anti-p24 is also a useful prognostic marker (Table XII). The relative hazard of the near normal groups (CD_4^+ T cells > 30% and positive anti-p24) was not equal to 1.0 because we used the intermediate group (CD_4^+ T cells 20-30% and weakly positiveanti-p24) as baseline for calculating the relative hazard. If we used the near normal group as baseline, the relative hazard of the very abnormal group (CD_4^+ T cells < 20% and negative anti-p24) would not be able to calculate

because of statistical reasons. This was also the same for the combination of absolute number of CD_4^+ T cells and anti-p24 (Table XII).

Goudsmit and colleagues have reported that circulating immune complexes can be detected in individuals with HIV antigenemia and that p24 antigen can be dissociated from the immune complexes[25]. The combinations of CD_4^+ T cells with either p24 antigen or anti-p24 seem to be better prognostic markers because it indicates both immune deficits (decreasing CD_4^+ T cells) and an increase in viral replication[113]. In fact, Fahey et at have reported the better prognostic markers when CD_4^+ T cells is combined with neopterin or β_2-microglobulin as compared to individual markers alone[88].

In summary, our studies indicate that the well described lymphocyte and serologic markers reported in the American and European HIV patients can also be applied to the Thai patients. This is particularly true for CD_4^+ cells count and p24 antigenemia either singly or in combination. The markers can either be used to support the clinical staging of the patient or to predict the progression to full-blown AIDS or death.

Other laboratory prognostic markers may soon become available for routine clinical use. These include levels of enhancing and neutralizing antibodies, CD_8 suppressive factor and the level of NEF (negative factor) gene product[1]. Until then, similar evaluations in Thai patients are needed to sort out the reliable, convenient and inexpensive markers or combinations of markers.

Conclusions :

1. The following immunologic abnormalities, namely, decreased CD_4^+ T cells, increased β_2-microglobulin level, decreased anti-p24 titer and increased p24 antigen level correlated well with the stages of HIV infection in Thai patients. Most profound changes were seen in AIDS, followed successively by ARC and normal or near normal in PGL or asymptomatic. Therefore, these immunologic markers may be used as laboratory adjuncts for making diagnosis of various stages of HIV infection in Thai patients. The degree of immunologic abnormalities at entry also correlated well with the advancement of the disease.

2. Initial values (i.e., values at the first diagnosis of HIV infection) of the number and percentage of CD_4^+ T cells, the number of CD_8^+ T cells, β_2-microglobulin level, p24 antigen level and anti-p24 could be reliably used as prognostic markers for HIV progression in our preliminary 2 year follow-up.

3. Adding p24 Ag to the measurement of CD_4^+ T cells seemed to substantially increase the prognostic value than either was used alone.

4. Our 2-year natural history follow-up study could eventually separate our patients into the progressor and the non-progressor groups based an their clinical courses. The annual rate of clinical progression was estimated to be 6.8 %. There were no differences in the age, sex or risk behaviors between the progressor and the non-progressor groups. Initial values of the immunologic markers, except β_2-microglobulin level and CD_4/CD_8 ratio, could not differentiate progressors from non-progressors. However, the difference in the immunologic markers between the 2 groups became obvious after a minimum of 12 months of follow-up.
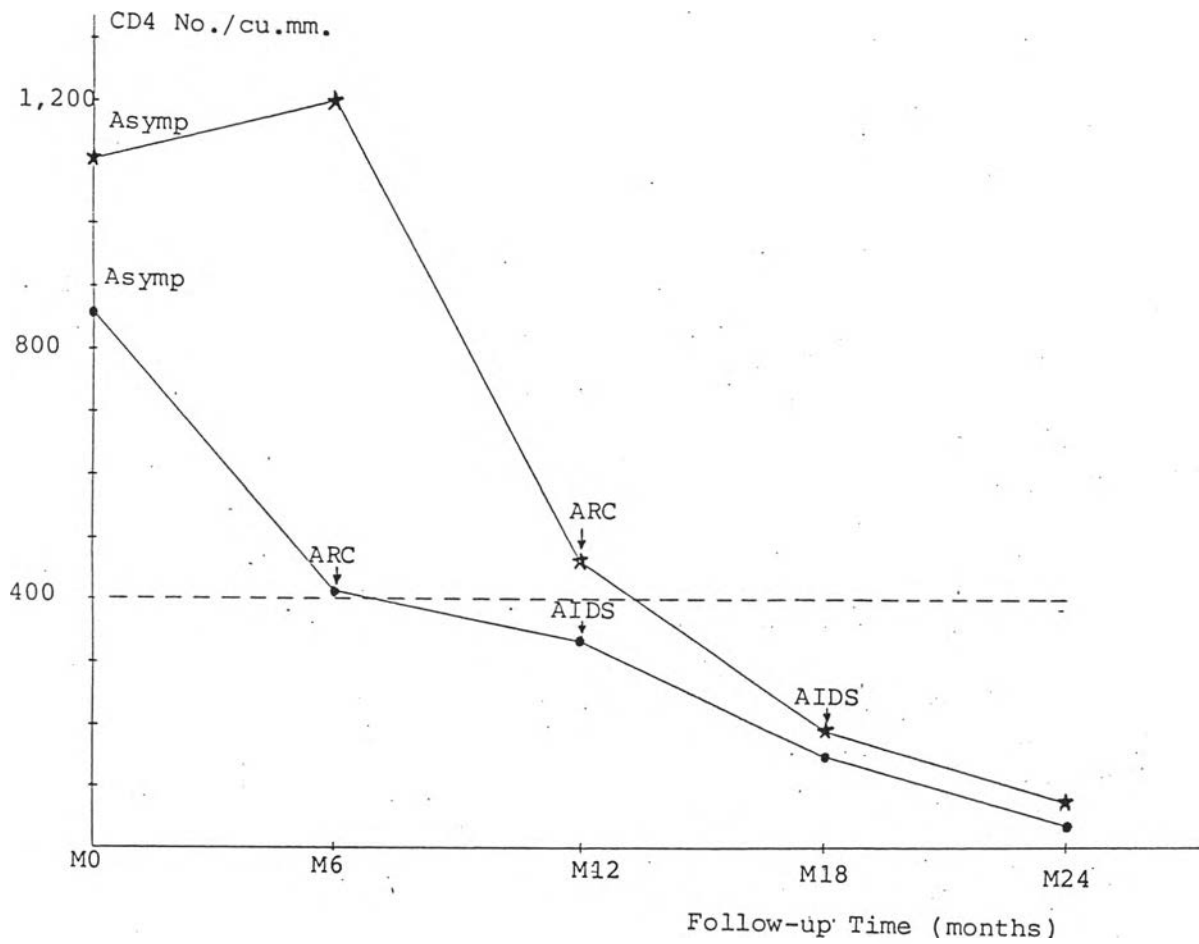
Figure 23. Sequential follow-up of absolute number of CD4+
T cells in 2 HIV-infected patients (*—*from
blood transfusion, •—• from heterosexual) who
progressed from asymptomatic infection to full-
blown AIDS in 2 years to illustrate the correlation
of decreasing CD4+ cell count and clinical
progression of disease.