

Fourteen patients were assigned to receive amiodarone and 23 placebo. Two patients (one in each group) died before the end of the study.

Detection of autoantibodies—None of the patients treated with placebo had or developed autoantibodies. On day 1 none of the patients treated with amiodarone had detectable autoantibodies. On day 7 three of the 13 surviving patients treated with amiodarone had detectable antithyroid microsomal antibodies, and on day 30 six had them (55%). Tests for all other autoantibodies yielded negative results in both groups.

Thyroid function—The table shows the serum concentrations of triiodothyronine, thyroxine, and thyroid stimulating hormone. No differences were seen in triiodothyronine and thyroxine concentrations between the two groups. A significant difference in concentrations of thyroid stimulating hormone between the two groups was noted on day 30 ($p < 0.05$). In three patients treated with amiodarone the concentration of thyroid stimulating hormone rose to the hypothyroid range (8.3, 8.6, and 8.8 mU/l). Two of these three patients had antithyroid antibodies.

Discussion

The finding of thyroid antibodies in 55% of patients after they had been taking amiodarone for 30 days suggests that this drug has a

toxic effect on the thyroid even when taken for fairly short periods. Previously such antibodies have been reported only in patients given long term treatment.³ Follow up evaluation six months after the withdrawal of amiodarone in the seven patients who had detectable antimicrosomal thyroid antibodies showed complete clearance of the autoantibodies. Similarly, concentrations of thyroid stimulating hormone in these patients reverted to normal. Thus the toxic effect seems to be reversible when the drug is used for short periods.

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(Accepted 18 November 1985)

Distinct IgG recognition patterns during progression of subclinical and clinical infection with lymphadenopathy associated virus/human T lymphotropic virus

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Abstract

Longitudinal IgG recognition patterns of viral proteins were studied in 15 men who had seroconverted for lymphadenopathy associated virus/human T lymphotropic virus (LAV/HTLV-III). Antibodies to the major viral core protein p24, which is a cleavage product of the gag gene encoded precursor protein pr55, appeared first. These were soon followed by antibodies to pr55 and more gradually by antibodies to the other gag gene encoded cleavage product p18, the env gene encoded transmembrane glycoprotein gp41, the env gene encoded glycoproteins gp65 and gp110, and the putative pol gene product p33.

In 13 subjects who remained healthy the reactivity to the different proteins increased or stabilised with time, while in two men who developed acquired immune deficiency syndrome (AIDS) the reactivity, most noticeably to gag encoded proteins, diminished before or at the onset of symptoms.

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Introduction

Lymphadenopathy associated virus (LAV),^{1,2} also designated human T lymphotropic virus type III (HTLV-III),³ is the causative agent of acquired immunodeficiency syndrome (AIDS). IgG antibodies to LAV/HTLV-III are found in nearly all patients with AIDS or signs preceding AIDS, whereas they are virtually absent in populations not at risk.^{4,7} Furthermore, LAV/HTLV-III can be isolated from many subjects with AIDS or signs preceding AIDS,⁸ and transmission of the virus by blood transfusion has led to the subsequent development of AIDS.^{9,10}

In a Swiss seroepidemiological study Schüpbach *et al* found evidence for the following pattern of IgG antibody response in human LAV/HTLV-III infection: in early and asymptomatic infection antibodies to the gag gene encoded protein p24^{11,12} predominate, whereas in later and symptomatic infection antibodies to the env gene product gp41^{11,12} are found most consistently, and with progression to AIDS antibody titre to p24 declines or disappears.¹³

We present data on the protein recognition patterns of LAV/HTLV-III in sequential serum samples from 15 individuals, two of whom eventually developed AIDS, that were followed from seroconversion to a maximum of three and three quarters years later.

Subjects and methods

Sequential serum samples obtained from 13 homosexual men who participated in a study of the efficacy of hepatitis B vaccine between 1980 and 1982¹⁴ and had seroconverted for LAV/HTLV-III¹⁵ were studied. Blood was collected at monthly intervals during the initial five months of the trial and every three months thereafter. In these 13 subjects the average time from the last serum sample that was negative for anti-LAV/HTLV-III to the first that was positive for anti-LAV/HTLV-III was 10.9 weeks (range 1-16 weeks). One subject developed AIDS three years after seroconversion. Six men entered a new prospective study on AIDS, which started in October 1984.

For the whole group the average length of follow up after seroconversion was 109.5 weeks (range 12-198 weeks).

Apart from this group two other subjects who had seroconverted were studied. One subject had seroconverted after receiving a blood transfusion and developed AIDS two and a half years later. The other person, whose clinical history has been described elsewhere, had a transient mononucleosis like illness two weeks after sexually acquired LAV/HTLV-III infection.¹⁶ Both subjects were followed up for 134 weeks after seroconversion.

IMMUNOBLOT ANALYSIS

Heat inactivated LAV/HTLV-III purified with a sucrose gradient was fractionated by electrophoresis using a 12% polyacrylamide slab gel in the presence of sodium dodecylsulphate.¹⁷ The protein bands on the gel were transferred to a nitrocellulose sheet (0.45 µm; Schleicher and Schuell,

and 2). Taking p24 recognition as point zero, we eventually recognised three other LAV/HTLV-III proteins in serum samples from all persons studied; pr55^{gag} from 12 weeks on, gp41^{env} from 40 weeks, and gp65^{env} from 78 weeks (fig 2). Serum samples from 13 subjects eventually reacted with p18^{gag} and p33^{pol} (fig 2). Pr110^{env} was recognised by serum samples from three subjects only, 20 weeks or more after seroconversion.

In one subject the precise time of LAV/HTLV-III transmission could be determined.¹⁶ Two weeks after a sexually acquired LAV/HTLV-III infection antibodies were not found, but four weeks after transmission his serum reacted (strongly) with p24, and later with other proteins.

PROGNOSTIC IMPORTANCE OF IMMUNOBLOT PATTERNS

In 13 subjects no serious persistent disease developed. The intensity of the protein bands either remained constant or increased in all these subjects

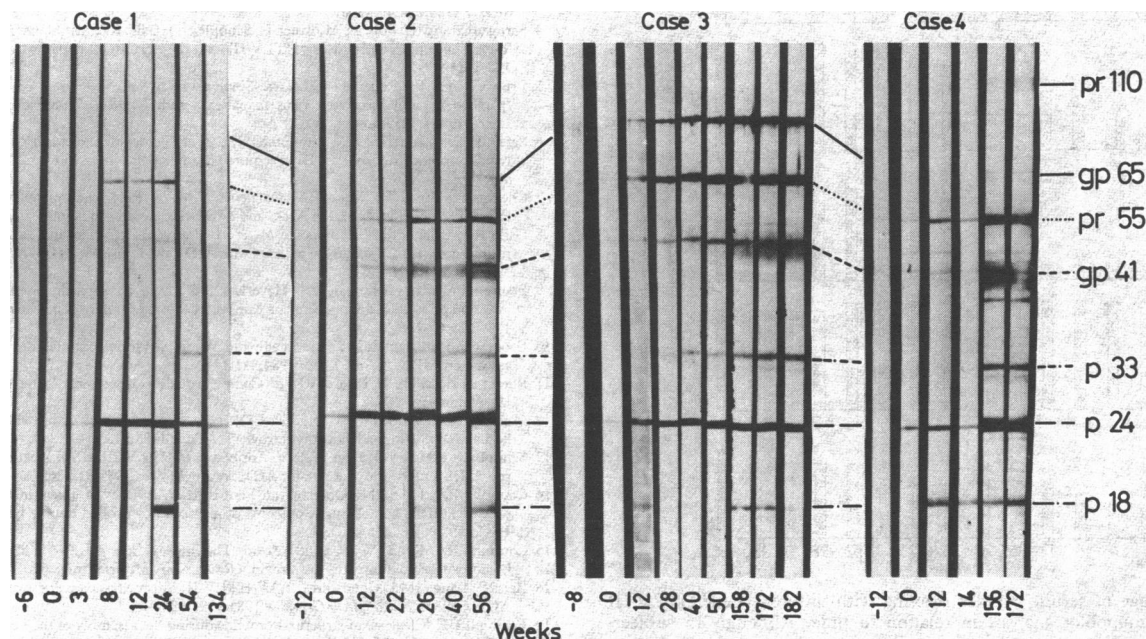


FIG 1—Immunoblot analysis of consecutive serum samples for antibodies to LAV/HTLV-III. Time of first immunoblot positive serum sample was taken as point zero. Subject in case 1 is a man in whom AIDS developed 130 weeks after seroconversion; subjects in cases 2, 3, and 4 are men without known lasting LAV/HTLV-III related symptoms.

Dassel, West Germany).¹⁸ After this transfer the sheet was rinsed with phosphate buffer saline and Tween 0.05% and incubated for two hours in phosphate buffer saline (pH 7.4) at room temperature. The sheet was then rinsed with phosphate buffer saline and Tween, dried, and stored at 4°C until use. When used the sheet was cut into strips and each strip preincubated for one hour at 37°C in 2.5 ml phosphate buffer saline and Tween containing 4% normal goat serum. Subsequently 25 µl of heat inactivated test serum was added to each strip separately, and incubation was continued for one hour at 37°C. After washing with phosphate buffer saline and Tween the strips were incubated at room temperature for one hour with biotinylated goat antihuman IgG (Vector, Burlingame, California). After another washing the strips were incubated for one hour at room temperature with a preformed complex of avidin and biotinylated horse radish peroxidase (Amersham International, Bucks). After washing colour was developed using 4-chloro-1-naphthol as substrate.¹⁹

Proteins p18^{gag}, p24^{gag}, and pr55^{gag} were considered to be encoded by the gag gene; proteins gp41^{env}, gp65^{env}, and pr110^{env} by the env gene,^{11 12}; and protein p33^{pol} by the pol gene.²⁰

Results

PROTEIN RECOGNITION PATTERNS OF LAV/HTLV-III

In all 15 subjects studied the first positive serum sample reacted with p24^{gag}. Reactivity against pr55^{gag} was also often detected at this time, whereas it usually took longer for the other proteins to be recognised (figs 1

throughout the observation period, even when this was longer than three years (cases 3 and 4 in fig 1).

In two subjects AIDS developed. One of these patient's clinical history has been described fully elsewhere.¹⁵ Two and a half years after seroconversion he developed AIDS related complex, and half a year later AIDS was diagnosed. At the time he developed AIDS related complex his immunoblot pattern did not show p24^{gag}, while the only other protein that was clearly present before this time, gp41^{env}, decreased in intensity but remained visible until his death over a year later. The second patient (case 1, fig 1) was a heterosexual man who received multiple blood transfusions in June 1982. Seroconversion for LAV/HTLV-III occurred in July 1982. Two years later malaise, fever, and weight loss developed. AIDS was diagnosed two and a half years after seroconversion when he developed candida oesophagitis. Examination of his last two serum samples, taken 54 and 134 weeks after seroconversion, showed a gradual diminishing in the intensity of the protein bands, especially of p18^{gag} and p24^{gag}.

Discussion

As found in studies of experimental infection in non-human primates²¹ and in humans,²² we found that the early antibody response against LAV/HTLV-III was directed predominantly against gag gene products (especially p24 and pr55) and followed gradually by a response directed against env gene products (especially gp41 and gp65) and the pol gene product (p33). The earliest IgG response was directed against p24^{gag}. In the subject in

whom the precise time of LAV/HTLV-III transmission was known the lag time between infection and recognition of p24^{gag} was between two and four weeks.

Not only did antibodies against p24^{gag} indicate the early phase of LAV/HTLV-III infection but they also provided information in later infection. In subjects without AIDS the intensity of the

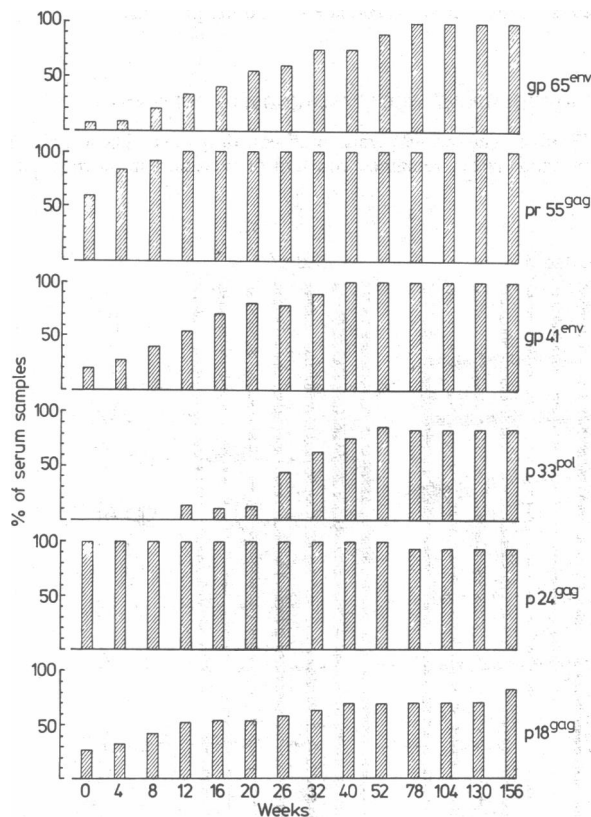


FIG 2—Percentage of serum samples reacting with different LAV/HTLV-III proteins by immunoblot analysis in relation to time. Although 15 subjects were studied not all were tested at each subsequent interval and not all were followed up for 156 weeks. At each interval shown, however, at least six subjects were tested.

response against all the different visible proteins stabilised or increased with time even when they were followed up for three years. As Biggar *et al* reported recently,²² we found in the two subjects in whom AIDS eventually developed that the intensity of the response against the different proteins started to diminish, most noticeably for gag encoded proteins, two and a half years and one year after seroconversion, respectively. In one of these patients total

eradication of p24^{gag} coincided with the onset of AIDS related symptoms, whereas gp41^{env} remained visible to his death.

Schüpbach *et al* tentatively concluded from transectional data that decline or loss of antibody titre to p24^{gag} signifies impending transition to AIDS¹³; findings from our longitudinal study support this. Whether the decline of anti-p24^{gag} titre is due to excessive production of viral antigen in progressive symptomatic LAV/HTLV-III infection or to exhaustion of B cell lines producing anti-p24^{gag}, or both, has yet to be investigated.

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(Accepted 9 December 1985)

100 YEARS AGO

A curious case of early puberty was shown by Mr. Bruce Clarke at the meeting of the Pathological Society on January 19th. A large muscular boy, apparently ten or twelve years old, came under treatment on account of bowing of the tibiae due to rickets, and the parents made the astonishing statement that he was born on May 17th, 1882, a statement subsequently confirmed by a reference to the birth-certificate preserved at Somerset House. He was three feet eight and a half inches high, and weighed four stone six pounds. There was some down on his cheek, and, though there was no hair on the chest or in the armpits, he was as hairy as a man about the pubes and in the perinaeum. His penis was as large as a man's, and was noticed to be erect every morning, though the testicles were rather smaller than those of an adult. The pomum Adami was well developed, and his voice was cracked like that of a boy losing his "childish treble." The development of his brain, however, had not kept pace with the growth of his body, and his mental state was about that of a child of his years. The girth of the head round the occipital and frontal protuberances was twenty-one inches, which is

certainly not very small for his bulk. It is interesting to note that, in spite of the great development of his sexual organs, he has never given any evidence of sexual desire, and that no seminal emissions are known to have occurred. The boy was the third child in a family of five, and he was bigger than the eldest child, who was over seven years old. He was suckled for nine months. At about one year of age he began to grow rapidly and to eat voraciously, so that nothing seemed to satisfy him. Hair began to grow on the pubes, and, before he was a year and a half old, he was as hairy as a man. At about that time this very rapid rate of growth ceased, and, since then, his mother thinks he has only grown with ordinary rapidity. Mr. Bruce Clarke performed osteotomy on the bowed tibiae, and the boy made a good recovery. A few cases are on record where puberty developed between the ages of two and three years, but Mr. Clarke informs us that he has been unable to find any other case recorded where it developed before eighteen months of age.

(*British Medical Journal* 1886;i:263.)