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Activated interferon system in healthy homosexual men

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Summary

More than 50% of a group of healthy homosexuals in Israel were found to have an activated interferon (IFN) system as evidenced by markedly elevated blood IFN levels, increased in vitro production of IFN by unstimulated peripheral blood mononuclear cells and HuIFN-α and HuIFN-γ production by appropriately stimulated cells, and a surprisingly high incidence of an antiviral state of cells. This pattern resembles that found in persons with acute viral illness, and is unlike that found in normal healthy controls. The type of IFN in the blood was found to be unusual in that it was mainly HuIFN-α, pH 2-labile, a type of IFN found in certain collagen diseases as well as in homosexual men suffering from Kaposi's sarcoma or lymphadenopathy. Natural killer (NK) cytotoxic activity was found to be somewhat lower than that found in normal controls, although no correlation was found between blood IFN levels and NK activity. Mean (2′–5′)-oligoisoadenylate synthetase levels in cell extracts were intermediate between normal controls and patients with viral illness. Likewise no correlation was found between enzyme levels and blood IFN levels.

The highly activated IFN system found in certain homosexuals, as well as the increased spontaneous production of IFN by unstimulated mononuclear cells, suggest the possibility of the presence of a virus, active or latent, in these individuals. This virus could be a retrovirus such as HTLV-III or LAV which have recently been isolated from AIDS patients. The special type of IFN present could be the response to a novel virus in an unusual situation. On the basis of recent reports, we speculate that homosexuals with highly activated IFN systems who produce pH 2-labile HuIFN-α could be at increased risk for developing AIDS.

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Introduction

Interferon (IFN) has been reported to be elevated in the serum of some homosexuals and haemophiliacs who subsequently developed an acquired immune deficiency syndrome (AIDS) [2,6]. In our ongoing studies of the IFN system in a variety of pathological and other conditions [12], we have now studied a group of healthy homosexuals in Israel in an attempt to learn more about the possible mechanisms involved in the development of AIDS.

AIDS is a newly discovered disease with a propensity for occurring in homosexuals [13], patients with haemophilia who receive repeated injections of pooled blood products [11], habitual intravenous drug users, and persons belonging to certain ethnogeographic groups such as those from Haiti [22] or Zaire [3]. Clinically, the syndrome presents as increased susceptibility to Kaposi's sarcoma or to certain opportunistic infections such as those caused by *Pneumocystis carinii*. Immunologically, the most pronounced abnormality is a decreased ratio of phenotypic helper (T_4) to suppressor (T_8) cytotoxic T-lymphocytes [13]. Once AIDS develops, mortality is high, although illness may be prolonged for months to years. As yet no treatment has proved effective. Epidemiological evidence led to the almost inescapable conclusion that a transmissible agent, probably a virus, was causally related to the epidemic [13]. In the past, several commonly known viruses were suspected, including Epstein–Barr virus (EBV), cytomegalovirus (CMV) [5] and hepatitis B [16]. However, at present it seems almost certain that a subgroup of retrovirus associated with human T-cell leukaemia (HTLV-III) [7,18,19] and a possibly related retrovirus associated with a lymphadenopathy syndrome (LAV) [1,17] are the etiological agents.

Our previous studies of the human IFN system response in health and disease have revealed significant differences between healthy individuals and those with viral infections [12]. In this study we report on IFN system assays in healthy homosexuals. Our results indicate that in a high proportion of them the IFN system is highly activated, as it is in viral illnesses, and unlike that found in non-homosexual healthy individuals.

Subjects and Methods

Sterile heparinised blood samples were taken from 110 healthy male homosexuals as part of a larger study of the immune status in these individuals. We cannot rule out an element of bias in the selection of our subjects, as they presented themselves for examination after a general announcement had been made of the aims of the study, and it is conceivable that those most concerned about their health were the first to respond and to be included in the population on which this preliminary report is

based. All were physically examined and detailed histories were taken. At the time of examination all subjects included in this study claimed to be healthy and denied the existence of viral or other illnesses. No individual was found with unexplained lymph node enlargement. (The clinical and immunological parameters of this study are to be reported separately).

Fresh whole blood samples from 57 of the subjects were placed on Ficol-paque (Pharmacia, Uppsala, Sweden) gradients and from each sample peripheral blood mononuclear cells (PBMC) were isolated for the study of in vitro IFN production, evaluation of the presence of a functional antiviral state, determination of natural killer (NK) cell activity, and assay of (2'-5')-oligoisoadenylate synthetase levels in cellular extracts. The plasma samples from these individuals as well as from the remaining 53 subjects were either examined immediately or stored at -70°C until used for assay of the IFN content and characterisation of IFN type, usually within 1-2 weeks.

Blood samples from normal healthy individuals, mainly doctors, students, nurses or laboratory staff, assayed over the years on a continuing basis in the interferon laboratory, constituted a 'normal' control group. 110 patients at the acute stage of a viral illness comprised a 'virus' control group. Samples from the latter two groups were in some cases assayed in parallel with those of the homosexuals when suitable 'virus' patients or controls were available on the day of sampling. Serum samples which had been previously stored were more often assayed in parallel. The patients and control group were not matched for age or sex, as we have found that these factors do not have a significant effect on the various parameters tested.

IFN assay

Plasma IFN levels in vivo and IFN production by PBMC in vitro were determined by immunoassays in which virus yield was measured by quantitating viral protein using antiserum to vesicular stomatitis virus (VSV) [23]. HeLa (H229) cells in 96-well tissue culture plates (Costar, Cambridge, MA, U.S.A.) were incubated with and without known amounts of standard HuIFN-α as well as with serially diluted plasma from test subjects. Following incubation, the cells were infected with 10^3 tissue culture infective doses ($TCID_{50}$) of VSV. Similarly, supernatants from PBMC cultures, either unstimulated or stimulated with polyinosinic-cytidilic acid (poly-IC) for HuIFN-α production and phytohaemagglutinin (PHA) for HuIFN-γ production, were assayed for IFN content [12]. Each assay was standardised by the inclusion of a laboratory standard calibrated against an international HuIFN-α standard (MRC Research Standard B69/19). Results are expressed in units/ml.

Characterisation of IFN in blood

The type of IFN present in the blood of 29 subjects found to have increased IFN blood levels was characterised by using antisera to the different classes of IFN as well as by evaluating the stability of the IFN at pH 2. Polyclonal antibodies prepared in rabbits against HuIFN-α and HuIFN-β and monoclonal antibodies to HuIFN-γ (Interpharm, Rehovot, Israel) had been previously calibrated for neutralisation of the corresponding IFN. From each subject tested 100 μl of plasma containing up to 100

units of IFN activity was added to an equivalent volume of each antiserum, or various combinations of antisera that would specifically neutralise up to 100 units of the corresponding IFN. The mixture was incubated at room temperature for 1 h and residual IFN activity was assayed. Diminution of activity by more than 50% was considered significant and indicative of the presence of the corresponding IFN [4]. In order to characterise IFN stability at pH 2, the pH of the plasma was adjusted to 2 by the addition of 1 N HCl and readjusted 24 h later to pH 7.4 with 1 N NaOH. In some cases aliquots of the sample were dialysed to pH 2 and dialysed back to pH 7.4 1 day later. IFN activity was determined before and after treatment, and the percent diminution of activity determined as above [4].

Assay of the antiviral state

The antiviral state was evaluated by measuring the replication efficiency of VSV in PBMC [12]. After pretreating PBMC with HuIFN-α at concentrations ranging from 0 to 128 units/ml, the cells were infected by exposure to 1000 $TCID_{50}$ of VSV. The virus yield was assayed by means of an ELISA assay after 48 h by measuring the amount of viral protein in culture. A virus yield greater than the original infecting dose indicated viral replication and absence of an antiviral state in the PBMC examined. This is a normal finding in most healthy people who have very little or no IFN in the blood. Our earlier studies have shown a good correlation between increased levels of IFN in the blood (>16 units/ml) and the presence of an antiviral state ($<10^3$ $TCID_{50}$ viral yield) in the PBMC [12].

Measurement of (2'–5')-oligoisoadenylate synthetase activity

This was assayed in PBMC extracts using ^{32}P-labelled α-ATP in a radioisotope assay, and the results expressed as nmols of ATP incorporated per 10^5 cells per h [20].

Evaluation of natural killer (NK) activity

NK activity was assayed using the ^{51}Cr release method. Effector cells (PBMC) were incubated in triplicate with ^{51}Cr-labelled K562 target cells in ratios of 20 : 1 and 40 : 1 for 3 h. A second set of assays was performed using PBMC pretreated overnight with 500 units/ml of HuIFN-α. As controls, labelled target cells ruptured with detergent (maximal release), and target cells in medium alone (minimal 'spontaneous' release) were used. Aliquots (100 μl) of the supernatants were tested for radioactivity in a gamma spectrometer. Cytotoxicity (%) was calculated by dividing the difference between the mean release from test samples and the spontaneous release, by the difference between the maximum release and the spontaneous release [9].

Lymphocyte subpopulations. These were evaluated using anti-T_3, anti-T_4 and anti-T_8 monoclonal antibodies (Becton-Dickenson, California, Anti-Leu series) in a fluorescence-activated cell sorter.

Statistical evaluation:

The significance of differences among subject groups was evaluated by Student's *t*-test.

Results

The assays performed in this study are intended to throw some light on the state of the IFN system at the time of blood sampling in healthy homosexuals, including the ability of IFN-producing blood cells to spontaneously produce IFN in culture or to respond in vitro to specific stimulants, and the extent to which, as a secondary effect, an individual's IFN is capable of activating certain immune mechanisms.

Table 1 summarises the results of these assays in normal healthy persons, in patients at the acute stage of a viral disease and in the present study group of healthy male homosexuals. It can be seen that there is some similarity between the results obtained in healthy homosexuals and in the virus disease group in that the IFN system was activated in both groups. On the other hand, there were pronounced differences between the results in healthy homosexuals and in the normal controls: whereas only 11% of normal controls had blood IFN levels in excess of 16 units/ml, and those at relatively low titres, 60% of homosexuals had greatly increased in vivo IFN values. Homosexual PBMC in culture without PHA or poly-IC stimulation spontaneously produced IFN equal to or in excess of 16 units/ml in 29 of the 59 samples tested, with levels reaching as high as 435 units/ml. With poly-IC stimulation, higher levels of HuIFN-α were produced by PBMC from homosexuals than from the other two groups ($P<0.01$); with PHA stimulation, higher values of HuIFN-γ were obtained with PBMC from homosexuals than from healthy individuals ($P<0.05$) or those with viral illnesses ($P<0.01$).

Even more remarkable are the results of the functional biological assay for the presence of an in vivo antiviral state in the cells. Of the 45 homosexuals studied, 42 (93%) had PBMC in an antiviral state, i.e. they did not support replication of VSV in vitro. These findings are in marked contrast to those in the normal control group (13%) and resemble the findings in the virus disease group (66%).

Table 2 records the types of IFN found in vivo in homosexuals. In 27 out of 29 cases (93%) from whom plasma or serum was available and in which IFN blood levels were greater than 60 units/ml, the IFN was characterised as HuIFN-α, and in 17 of them

TABLE 1

Evaluation of the IFN system in healthy homosexuals

Assay	Healthy persons	Patients with viral illness	Healthy homosexuals
Plasma IFN (units/ml)	5 (75; 2.3–7.7)[a]	57 (110; 40–74)	165 (110; 90–240)
IFN-α production by PBMC	382 (69; 260–504)	245 (103; 163–326)	743 (57; 536–455)
IFN-γ production by PBMC	170 (60; 119–220)	192 (101; 142–241)	494 (57; 296–691)
IFN-production by non-stimulated PBMC	2 (68; 0.8–3.5)	11 (98; 6–16)	46 (57; 19–73)
% Patients with cells in antiviral state	13 (60)[b]	66 (96)	93 (45)

[a] Logarithmic mean (n; 95% confidence interval).

[b] In parentheses: n = number.

TABLE 2

Characterisation of IFN in serum from homosexuals

Treatments	Antisera against HuIFN ($n = 29$)			pH 2 ($n = 18$)
	IFN-α	IFN-β	IFN-γ	
% Reduction in titre (± S.D.)	85 (± 20)	24 (± 31)	10 (± 13)	85 (± 19)
% Samples with >50% reduction in titre	96	21	0	94
% Samples with >80% reduction in titre	79	7	0	72

who were further studied, all were pH 2-labile (>50% neutralisation or diminution of titre at pH 2). In only two cases was there no significant neutralisation of the antiviral activity by anti-HuIFN-α antibody. In 79% of cases, the diminution of titre following treatment with anti-HuIFN-α antibody was in excess of 80%, and in half the cases it was more than 90%. With anti-HuIFN-β antibody complete neutralisation occurred in one of the 29 and between 60% and 80% diminution of titre in five others. Of these six individuals, one was found to have only HuIFN-β; the other five had both HuIFN-α and HuIFN-β. None of the 29 had significant amounts of HuIFN-γ in the serum, although 20% to 40% neutralisation by anti-HuIFN-γ antibody was seen in six individuals.

NK activity in normal controls and in healthy homosexuals is recorded in Table 3. Although the normal controls showed slightly higher mean activities at both ratios of effector to target cells (20 : 1 and 40 : 1), the differences were not statistically significant. Preincubation of effector cells with HuIFN-α in vitro increased the mean activity in normal controls at the 20 : 1 ratio by 35%, in homosexuals by 43% and in the virus control group by 88%. In vivo blood levels of IFN did not correlate with NK activity either with or without added exogenous HuIFN-α. Furthermore, the in vitro response to exogenous IFN could not be correlated with the IFN levels in the blood. Synthetase levels in homosexuals were not statistically different from those found in normal controls (Table 3). Likewise no consistent correlation was found between the IFN levels in the blood and levels of synthetase in PBMC extracts ($r = 0.163$).

Table 4 presents a comparison between total lymphocyte and T-cell populations in two groups of homosexuals: the first consisted of the 15 individuals with the highest IFN blood levels (mean = 920 ± 688 units/ml), and the second of 15 individuals in whom the IFN system was minimally or not activated (mean = 14 ± 16 units/ml). No difference was found between the two groups with regard to the total number of lymphocytes or of the ratio of helper to suppressor cells: however, the mean numbers of helper and suppressor cells in both groups were lower than those found in normal controls.

TABLE 3

IFN-related activities in homosexuals

Group	NK activity (mean ± S.D.) at E:T ratio					(2'-5')-Oligoisoadenylate synthetase	
	n	20 : 1		40 : 1		*n*	nmol αATP/ 10^5 cells per h
		Unstimulated	+ IFN	Unstimulated	+ IFN		
Homosexuals (% increase)	44	14 ± 10	20 ± 13 (43%)[a]	22 ± 14	32 ± 16 (45%)	27	1.38 ± 1.22
Normal controls (% increase)	13	20 ± 10	27 ± 10 (35%)	34 ± 12	41 ± 10 (21%)	19	1.14 ± 0.7
Viral illness (% increase)	27	17 ± 13	32 ± 17 (88%)	25 ± 15	42 ± 18 (68%)	19	1.76 ± 1.18

[a] % increase over unstimulated in parentheses.

TABLE 4

T-cell subpopulations in homosexuals with activated vs. non-activated IFN systems

	Activated (n = 15)	Non-activated (n = 15)
Blood IFN level (units/ml)	920 ± 688[a]	14 ± 16
Total lymphocytes (/cmm)	1957 ± 684	1667 ± 445
T-Helpers (T_4) (/cmm)	546 ± 232	564 ± 292
T-Suppressors (T_8) (/cmm)	399 ± 155	372 ± 143
T_4/T_8 ratio[b]	1.4	1.56

[a] Mean ± S.D.

[b] Mean ratio in controls: 1.58.

Discussion

The highly activated IFN systems found in many healthy homosexuals, with in vivo blood levels in some cases reaching as high as 2500 units/ml, resemble those found in patients with active viral infections [12]. However, all of our homosexual study cases denied symptomatology of current viral illnesses, and physical examination revealed no lymphadenopathy or evidence of acute viral disease except for what appeared to be a nonspecific urethritis in one or two cases. This suggests the possibility that the activated IFN system could be due to a chronic, smouldering or 'hidden' viral infection, although it does not rule out other possibilities such as the presence of an autoimmune reaction which in itself could be triggered by viruses.

It is well established that many viruses can remain inactive within cells for extended periods [21]. The question arises whether these 'hidden' intracellular viruses could be the stimuli for protracted IFN production. This possibility is supported in the present study by the finding of a significantly increased incidence of spontaneous or unstimulated IFN production by washed PBMC from homosexuals following 24 h culture in medium alone. In the normal controls unstimulated IFN production by PBMC cultures was rarely found, and when present it was usually below 16 units/ml (Table 1). Spontaneous production of IFN in culture has been reported in normal adults and newborns (and characterised as HuIFN-γ) [15], in cases with viral infections, and in some patients with cancer and autoimmune diseases [8]. In the two latter instances viruses have been considered possible etiologic agents. It is conceivable therefore that a persistent, non-symptomatic intracellular viral infection could be the cause of a chronically activated IFN system. It is possible too that this antiviral defence mechanism could break down later for reasons as yet unknown. It has been reported that AIDS patients develop a diminished capacity to produce IFN in vitro [14]. We found that the IFN system was totally paralysed in a patient with AIDS whose blood was assayed just prior to death; there was no IFN in the blood and no production of HuIFN-α or HuIFN-γ by stimulated PBMC in vitro. Although CMV, EBV or hepatitis B virus infections are common in homosexuals and in AIDS patients, it

seems unlikely, on the basis of our present knowledge, that these viruses are the primary cause of AIDS. However, one cannot rule out the possibility that they may act as triggers for activating other novel viruses which may be more directly involved in the etiology of AIDS. Recently, a human T-cell leukaemia retrovirus (HTLV-III) has been isolated from patients with AIDS and pre-AIDS [7,18]. Serum samples from 88% of patients with AIDS and from 79% of homosexual men with signs and symptoms that frequently precede AIDS, but from less than 1% of heterosexual subjects, were found to have antibodies reactive against antigens of HLTV-III [19]. In another study from France a lymphadenopathy-associated retrovirus (LAV), probably related to HLTV-III, has been isolated from patients at risk for AIDS suffering from a lymphadenopathy syndrome [1,17]. It seems most likely that these viruses are causally related to the development of AIDS, and therefore one might speculate that in some homosexuals they are present in an inactive form and could be responsible for activation of the IFN system.

We have previously reported that patients with viral infections and increased blood IFN levels had lower levels of in vitro HuIFN-α and HuIFN-γ production in appropriately stimulated PBMC than those found in healthy people with lower levels of blood IFN. We speculated at that time that a negative feedback mechanism, induced by increased levels of IFN in the blood, may cause suppression of IFN production by stimulated PBMC [12]. However, in our homosexual study group, production of HuIFN-α and HuIFN-γ in vitro by stimulated PBMC was greater than in the normal controls. This raised the question of whether the IFN in homosexuals is an unusual type of IFN which may not be able to trigger the feedback mechanism. We therefore proceeded to characterise the type of IFN present in homosexuals by using type-specific antibodies to HuIFNs as well as by evaluating acid lability. Almost every sample was found to consist of HuIFN-α which was pH 2-labile (Table 2). Few individuals had mixtures of HuIFN-α (major component) and HuIFN-β (minor component), and a single one had HuIFN-β alone. In no case did we find significant amounts of HuIFN-γ. The results of assays in which these antisera were used singly or in combination suggest that the IFN present in these individuals may consist of a mixture of the different types, the major portion being pH 2-labile HuIFN-α. The presence of multiple IFNs has been reported in patients with either systemic lupus erythematosus or vasculitis [10]. In a recent report, 52 out of 76 healthy homosexuals were found to have IFN in the serum (mean titre 31 units/ml), and in cases where the titre was greater than 64 units/ml, neutralisation studies showed that HuIFN-α was present in all cases, whereas HuIFN-γ was detectable in only two individuals [2]. In another study of homosexual men suffering from Kaposi's sarcoma and lymphadenopathy, increased blood IFN levels were found to be due to the presence of an unusual acid-labile HuIFN-α, similar to that found in patients with systemic lupus erythematosus [4]. However, in this study, only 8% of healthy homosexuals had detectable IFN activity in their serum. Of great interest was the report of a study of 76 homosexuals, in which the five with the highest blood IFN levels developed AIDS within 2–17 months of serum examination [2]. This finding led to the suggestion that the presence of increased levels of IFN in the blood of healthy homosexuals may be an early indication of the development of AIDS [2].

A good correlation has been reported between raised IFN blood levels and increased (2′–5′)-oligoisoadenylate synthetase levels in PBMC [20]. We could find no such correlation in homosexuals, a finding which raises the question as to whether the unusual HuIFN-α in the blood of these individuals is a poor inducer of synthetase in the cells. Since 93% of the homosexuals in this study had cells in an antiviral state, it appears from our results that increased levels of intracellular synthetase are not essential for the development of a cellular antiviral state as evaluated by our assay. We also found somewhat diminished NK activity in homosexuals compared to that in normal controls (Table 3), whilst preincubation of the cells with HuIFN-α increased cytotoxicity in all groups. Thus the presence of IFN in the blood (mainly HuIFN-α pH 2-labile), or its absence, does not appear to affect NK activity or its response to exogenous HuIFN-α added in vitro.

It has been suggested that AIDS develops following a breakdown of the immune mechanism, as evidenced by diminished number of T-helper cells as well as by diminished T_4/T_8 ratios in homosexuals, and that IFN production increases as a result of increased infections due to this immune deficiency. To test this hypothesis we examined two subgroups in our study population, one with highly activated IFN systems, the other with non-activated systems, and could find no significant differences in lymphocyte subpopulation numbers and T_4/T_8 ratios between the two groups (Table 4). The reason for the breakdown of the cellular immune system in these individuals, with the subsequent development of various opportunistic infections or of Kaposi's sarcoma with its inevitably fatal outcome, is currently under intensive investigation.

Note added in proof (received 31 January 1985)

The homosexual patient with the highest plasma IFN level (2264 U/ml) developed AIDS within a year of the assay. The disease first manifested itself as a *Pneumocystis carinii* infection which did not respond to the usual therapy. Subsequently, CMV infection also developed. The patient died 18 months after the first assay, when the IFN system study indicated a defective response with almost no production of IFN-α or IFN-γ by stimulated PBMC (see Table 5). Antibody studies, performed in retrospect on the patient's serum at the height of IFN-system activation by Dr. Ben Yishai, Rambam Hospital, Haifa, showed high titre antibodies to both HTLV-I and HTLV-III. We previously reported another terminal AIDS patient with a completely defective IFN system response [24]. It seems possible that the activated IFN system is an indication of an early persistent viral infection and that, as the disease progresses to clinical AIDS with immune suppression, the IFN response becomes deficient. At this stage treatment with IFN may be appropriate.

TABLE 5

Follow-up on the homosexual with the highest plasma IFN level

Dates	Plasma IFN level (U/ml)	IFN production by PBMC (U/ml)			PBMC antiviral state
		Non-stimulated	Stimulated		
			IFN-α	IFN-γ	
22/05/83	2,264	60	2,114	1,368	+
27/10/83	725	803	230	652	+
24/11/83	301	23	235	142	+
03/01/84	0	0	128	384	+
18/09/84	3	0	4	18	-
11/12/84		(Died from AIDS)			

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