with mammalian type C viruses by low-stringency blot hybridization or comparative nucleotide sequence analysis. In contrast, the HLM-2 *pol* gene showed appreciable homology not only with type B retroviruses but with type A, avian type C, and type D retroviruses as well. These findings are consistent with the known functional similarities in the requirement for divalent cations for each of these viral polymerases as well as with other studies indicating genetic relations between their *pol* genes (*5*).

The HLM-2 genome represents a mosaic of sequences characteristic of different retrovirus classes. It has *env* gene sequences most closely related to the type A virus, LTR sequences most homologous with the type D virus, and *pol* gene sequences related to each of these as well as to mammalian type B and avian type C viral genomes. Although HLM-2 also showed distant homology with HTLV-I in its *pol* region, HTLV-I is not endogenous to human cells (*15*) but is transmitted horizontally as an infectious, tumor-inducing virus of humans (*16*). Whether the HLM-2 human retroviral-related sequences are expressed or are etiologic agents of human neoplasia is unresolved.

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Naturally Occurring Antibodies Reactive with Sperm Proteins: Apparent Deficiency in AIDS Sera

Abstract. *A set of naturally occurring immunoglobulin M (IgM) antibodies that are reactive with a defined subset of proteins in the acrosomal cap region of human sperm has been identified. These antibodies are present in a broad spectrum of human sera from males and females, 1 day to 40 years of age, and are absent or markedly deficient in a large proportion of sera from individuals with the acquired immune deficiency syndrome (AIDS) or at risk for AIDS. The subset of proteins with which the IgM antibodies are reactive includes a factor (or factors) capable of inhibiting lectin-induced T-lymphocyte proliferation. The prevalence of the sperm-reactive IgM antibodies indicates that they are not elicited by sperm. Further, immunoreactivity of the sperm proteins resulting in depletion of specific circulating IgM antibodies, or other interactions between the sperm proteins and elements of the immune system, may be a factor in the suppressed state of the immune system in AIDS.*

In the course of our studies on the proteins of mammalian spermatozoa (*1*), we have identified a set of immunoglobulin M (IgM) antibodies in human serum that, although immunologically reactive with defined components of human sperm, appear to be normal constituents of circulating Ig's. This inference is based on the finding that these antibodies are present in more than 99 percent of

Table 1. Presence or absence of IgM antibody reactive with acrosomal cap region of human sperm in sera from individuals with and without (control) AIDS or ARC.

Group	Status or type of donor	Sera examined (number)	Sera positive for IgM antibody* (number)	Sera negative for IgM antibody*	
				Number	Percent
	Homosexual males				
AIDS or at risk for AIDS	No symptoms	14	11	3	21
	ARC	45	30	15	33
	AIDS	20	12	8	40
	Others†				
	No symptoms	9	8	1‡	
	ARC	3	3	0	
	AIDS	1	0	1‡	
	Total	92	64	28	
Control	Adult males§	45	44	1	
	Adult females§	35	35	0	
	Hospitalized adults‖	21	21	0	
	Hospitalized children¶	30	30	0	
	Total control	131	130	1	

*IgM antibodies reactive with acrosomal cap region of human sperm (see Fig. 1). †Intravenous drug users, hemophiliacs, and infants born to women with AIDS. ‡Female prostitute. §Sera obtained from routine medical examination (sexual preference not known.) ‖From miscellaneous hospital admissions (for diseases other than of the immune system). ¶Ages, 1 day to 2 years; hospitalized for other than immune system diseases.

the sera from healthy males and females ranging in age from 1 day to 40 years and from those hospitalized for other than diseases of the immune system (Table 1).

The possibility that antigens derived from sperm may have a role in the pathogenesis of the acquired immune deficiency syndrome (AIDS) in homosexual men has been proposed, with the expectation that elevated titers of sperm-specific antibodies would be detectable in the sera of these individuals (*2*). However, rather than elevated titers, we have detected absence or marked deficiency of the naturally occurring, sperm-reactive IgM antibodies in sera of 40 percent of patients diagnosed with AIDS, 33 percent of patients with AIDS-related complex (ARC), and 21 percent of homosexual men at risk for AIDS but without symptoms of the disease (Table 1).

The relative specificity of the IgM antibody deficiency is indicated by the observation that the total concentration of IgM antibody in these sera is within the normal range. Further, serial specimens of serum from each of two patients showed a distinct correlation between absence of the IgM antibodies and deterioration of the immune system. These data suggest a relation between the immune-suppressive aspect of AIDS and its prodromes and deficiency of antibodies reactive with, but not likely to have been elicited by, sperm.

The more general implication of these observations is that specific subsets of antibodies in human serum may have a role in immune regulation and may be vulnerable to challenge by foreign proteins bearing epitopes with which the antibodies are reactive. Accordingly, we have attempted to characterize the subset of sperm-reactive IgM antibodies present in normal and deficient in AIDS-related sera and of the molecular entities of human sperm with which the antibodies are reactive. A semen specimen from either of two donors with a high count of motile, morphologically normal sperm was used for cytologic study (*3*), and pooled specimens from eight donors were used for biochemical studies. All control sera (Table 1) were examined for reactivity with human sperm smears by indirect immunofluorescence with heterologous antibodies to the human M, G, and A isotypes (*4*). When assayed in this manner, the sperm neck and tail were stained nonspecifically and with variable intensity by each Ig antibody. However, the acrosomal cap region of the head was stained only with the IgM antibody; the postacrosomal region of the head did not stain (Fig. 1, A, B, and C). Staining of the acrosomal cap region with this antibody was abolished by treating the sperm with Triton X-100 (Fig. 1D). Staining of the neck and tail regions with any of the Ig antibodies was not abolished by this treatment. The characterization of the set of antibodies reactive with sperm heads as IgM was confirmed by a "double sandwich" immunofluorescence procedure with monoclonal antibodies to human IgM or IgG (*5*). Thus, human serum appears normally to contain a set of antibodies, specifically characterized as IgM, that bind to moieties in the acrosomal cap region of human spermatozoa. The proteins bearing those reactive epitopes are soluble in Triton X-100.

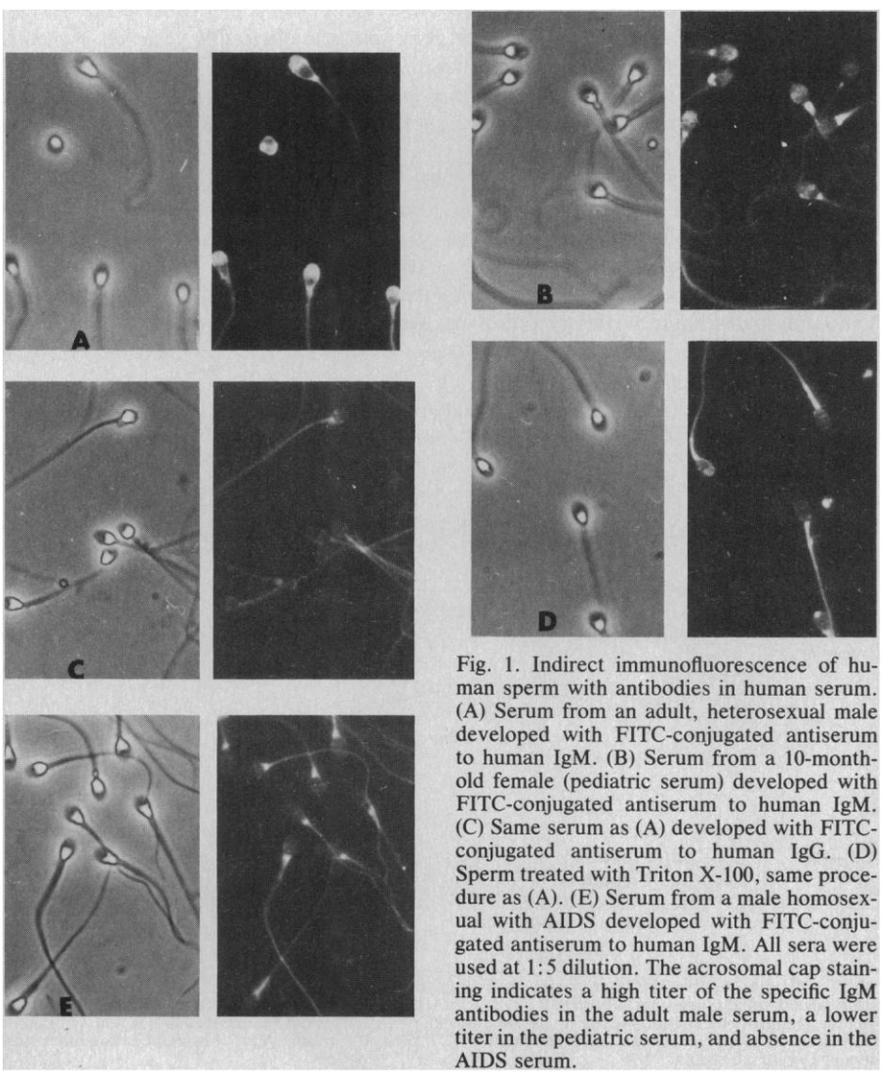

Fig. 1. Indirect immunofluorescence of human sperm with antibodies in human serum. (A) Serum from an adult, heterosexual male developed with FITC-conjugated antiserum to human IgM. (B) Serum from a 10-month-old female (pediatric serum) developed with FITC-conjugated antiserum to human IgM. (C) Same serum as (A) developed with FITC-conjugated antiserum to human IgG. (D) Sperm treated with Triton X-100, same procedure as (A). (E) Serum from a male homosexual with AIDS developed with FITC-conjugated antiserum to human IgM. All sera were used at 1:5 dilution. The acrosomal cap staining indicates a high titer of the specific IgM antibodies in the adult male serum, a lower titer in the pediatric serum, and absence in the AIDS serum.

Sera of AIDS patients and of individuals at risk for AIDS were examined by the immunofluorescence procedure and showed variable staining of the sperm neck and tail regions, but the IgM staining of the acrosomal cap region was absent as noted in Table 1 (see Fig. 1E). Immunoreactivity was estimated on the basis of intensity of fluorescence at serum dilutions of 1:5 to 1:40; a negative designation was made when there was no acrosomal cap staining at a serum dilution of 1:5 (Fig. 1E). Intensity of fluorescence was generally lower in sera from children (see Fig. 1, A and B); however, there was no ambiguity of designation of a serum as negative because any acrosomal cap fluorescence, even at low intensity, was accentuated by the absence of staining in the postacrosomal region. All negative and 10 percent of the positive sera (Table 1) were reexamined in a blind fashion; no discrepancy was observed.

The results of the immunofluorescence procedure were confirmed by immunoblots of electropherograms of sperm head proteins that were soluble in Triton X-100 (*6*). Sera designated as positive by immunofluorescence were reactive with a series of polypeptides in the molecular weight range of 29 to 95 kilodaltons, representing less than 10 percent of the bands on the stained electropherogram (Fig. 2, lane 2). Reactivity was displayed on the immunoblot with

the IgM antibody and not with the IgG antibody. Sera designated as negative by immunofluorescence showed no reactivity on the immunoblots (see Fig. 2, lane f). With the exceptions noted (Fig. 2), the positive sera of all subjects (Table 1) were reactive with the same bands. We conclude, therefore, that a discrete set of IgM antibodies normally present in human serum binds to epitopes on a specific subset of proteins from human sperm.

Total concentrations of circulating IgM in sera from AIDS patients or those at risk for AIDS that were deficient in the specific IgM antibodies reactive with sperm head proteins were within the limits of the normal range (*7*), which is consistent with previous reports (*8*) that isohemagglutinin as well as IgM titers are normal or elevated in sera of AIDS or ARC patients. These observations suggest that the deficiency represents depletion of specific classes of IgM antibodies rather than an overall impairment of IgM production.

The possibility that the presence or absence of the sperm-reactive IgM antibodies may be related to the severity of the immune-deficient state of the patient is supported by two case histories (Fig. 3). In case 1, the initial serum specimen, taken from a homosexual male with ARC, was positive for the IgM antibodies reactive with the acrosomal cap region of sperm heads (Fig. 3A). The second serum specimen, taken 11 months later when a diagnosis of AIDS with *Pneumocystis carinii* was established 1 month before the death of the patient (*9*), was devoid of those antibodies (Fig. 3B). In case 2, a serum specimen from a homosexual male with ARC contained no IgM antibodies reactive with the acrosomal cap region of sperm heads (Fig. 3C). A specimen taken 1 month later was still negative (Fig. 3D). The clinical and immunologic status of the patient, however, showed progressive improvement (*10*), and a serum sample taken 6 months after the first signs of remission showed that the IgM antibodies reactive with sperm heads were restored (Fig. 3E). Thus, the decline in the clinical status of patient 1 was correlated with depletion of the antibodies, and clinical improvement in patient 2 was correlated with restoration of the antibodies.

Studies were carried out in vitro to determine whether the sperm proteins recognized by circulating IgM antibodies (Fig. 2) display immunomodulatory properties. A fraction of sperm head proteins soluble in Triton X-100 was chromatographed on a Sephadex G-50 column by elution with phosphate-buffered saline (PBS; *p*H 7.2), and the fractions were tested for effect on phytohemagglutinin (PHA) stimulation of T-cell mitogenesis (Fig. 4). A marked inhibitory effect by fractions 50 to 60 was observed. Repeated experiments with various quantities of the fraction of sperm head proteins soluble in Triton X-100, examined for both PHA- and pokeweed mitogen–induced DNA synthesis confirmed this observation.

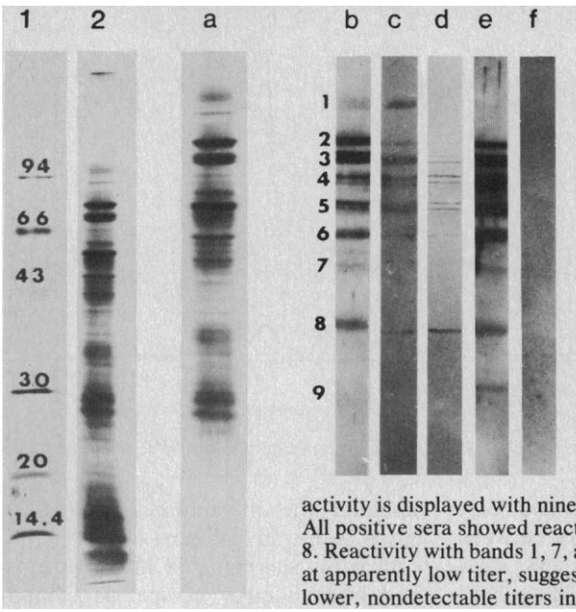

Fig. 2. (Lane 1) Molecular weight markers; (lane 2) SDS–polyacrylamide gel electrophoresis of fraction of sperm head proteins soluble in Triton X-100; (lane a) enlarged excerpt of lane 2. (Lanes b to f) Immunoblots with human sera and peroxidase-conjugated antibody to human IgM. (Lane b) Serum from a normal female; (lane c) serum from a normal heterosexual male; (lane d) pediatric serum; (lane e) serum from a homosexual male with AIDS (antibody-positive; see Table 1); (lane f) serum from a homosexual male with AIDS (antibody-negative; see Fig. 3B). All sera were used at 1:50 dilution. Reactivity is displayed with nine polypeptide bands (or doublets). All positive sera showed reactivity with bands 2, 3, 4, 5, 6, and 8. Reactivity with bands 1, 7, and 9 was detectable in some sera at apparently low titer, suggesting that it may be present at still lower, nondetectable titers in the others.

Because proteins were also distributed in other fractions of the chromatogram, it was relevant to determine which fractions included the antigenic moieties recognized by the circulating IgM antibodies and whether concordance of antigenicity and inhibition of T-cell mitogenesis could be shown. The fractions were segregated into seven pools (Table 2), the protein content of each pool was determined, and the immunoreactivity of two normal adult sera with each pool

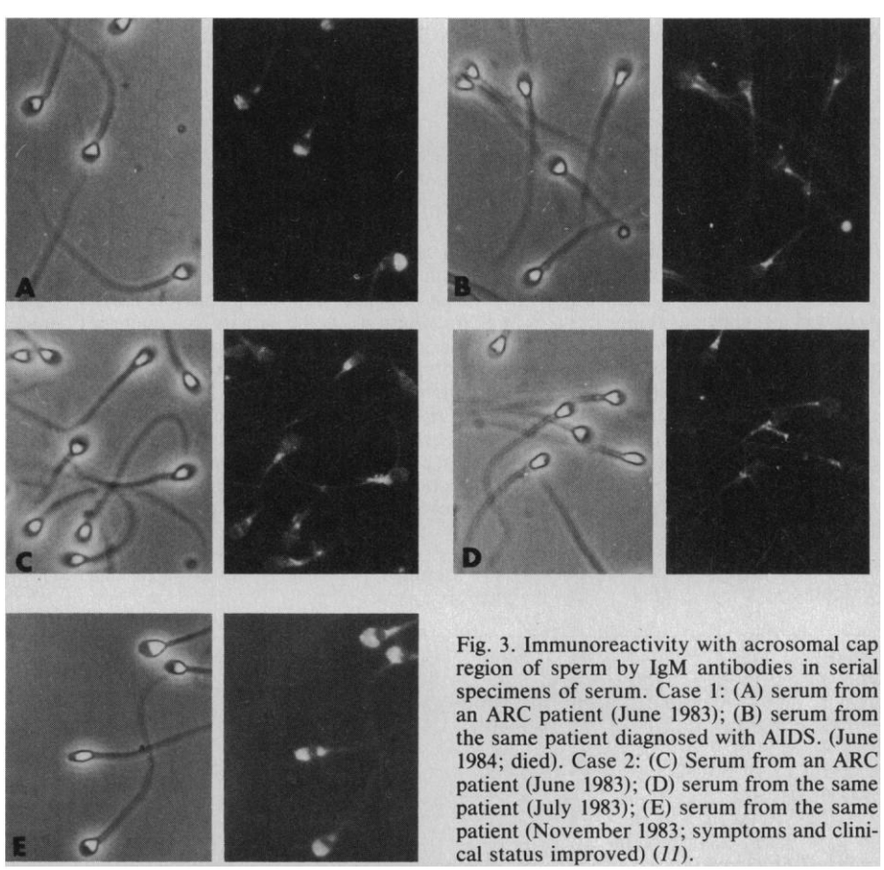

Fig. 3. Immunoreactivity with acrosomal cap region of sperm by IgM antibodies in serial specimens of serum. Case 1: (A) serum from an ARC patient (June 1983); (B) serum from the same patient diagnosed with AIDS. (June 1984; died). Case 2: (C) Serum from an ARC patient (June 1983); (D) serum from the same patient (July 1983); (E) serum from the same patient (November 1983; symptoms and clinical status improved) (*11*).

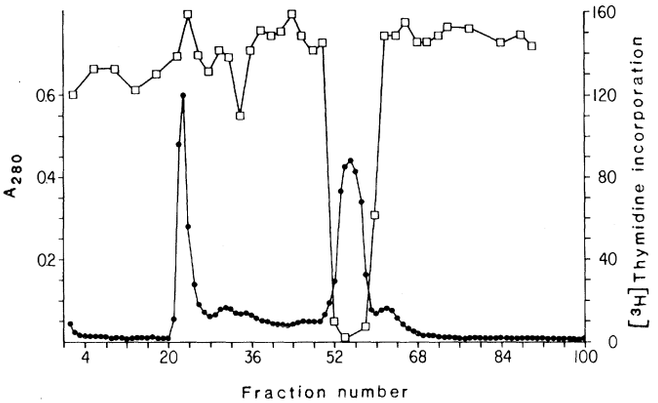

Fig. 4. Proteins of sperm heads soluble in Triton X-100, chromatographed on a Sephadex G-50 column. The 100 fractions were tested for inhibition of PHA-stimulated T-lymphocyte mitogenesis as described (*15*). Symbols: (□) [^{3}H]thymidine incorporation (with no sperm protein added, specific activity, 140×10^3 count/min); (●) protein concentration.

was assayed (Table 2). The major part (~90 percent) of the reactivity between the fraction of sperm head proteins soluble in Triton X-100 and the IgM antibodies of human sera was due to the proteins segregated in fractions 50 to 60 (Fig. 4). Thus, a subset of proteins in the acrosomal cap region of human sperm, which includes a factor (or factors) displaying an immunomodulatory property in vitro, is reactive with a subset of IgM antibodies that appear to be components of the normal set of circulating antibodies.

Considerable evidence has linked a human T-cell leukemia virus–lymphadenopathy-associated virus to the etiology of AIDS (*11*). However, although antibodies to one or another of the variants of that virus are detectable in sera of more than 30 percent of symptom-free homosexual men and more than 80 percent of those with ARC, only a small proportion of those proceed to AIDS (*12*). Thus, as is frequently stated (*13*), the epidemiological data suggest that the assault by the virus requires a predisposing or cooperative factor.

Because spermatozoa are characterized by a number of specific components, many of which are formed de novo after the individual has attained sexual maturity, sperm-specific moieties are believed to have a high potential for immunogenicity. During anal intercourse, sperm are introduced into milieux where that potential may be maximized (*14*), and some investigators have sought to identify sperm antibodies in the sera of homosexual men at risk for AIDS as evidence of an immunogenic cofactor in the disease (*2*).

In view of our data, a distinction should be made between antibodies elicited by sperm and antibodies immunologically reactive with, but not elicited by, sperm moieties. We have identified a set of IgM antibodies that are clearly of the latter class. Their distribution in the general population (Table 1) suggests that these antibodies are not the result of sperm-induced immunity but are normal constituents of human serum and thus may be components, possibly regulatory, of the immune system.

A relation between deficiency in those IgM antibodies and impairment of the immune system is shown in Table 1 and Fig. 3, and immunologic reactivity between a discrete subset of IgM antibodies and a specific subset of sperm-derived proteins is shown in Fig. 2. A dichotomy is suggested because sperm moieties may be implicated in the pathogenesis of AIDS. Thus, one hypothesis suggested by these observations is that certain IgM antibodies may have a role in maintaining immunologic homeostasis, and the introduction or an overload of the proteins with which a specific set of IgM antibodies are reactive may result in disruption of an equilibrium. The immunogenic factor in AIDS, therefore, might be depletion of a critical set of IgM antibodies, thereby establishing a medium in which the AIDS-associated retrovirus may be effectively pathogenic. An alternative hypothesis would take into account the demonstrated T-cell mitogenic inhibitory property of the sperm proteins. An overload of these proteins could suppress proliferation of a specific fraction of T cells, leading to an immunodeficient state.

The question then arises as to whether a deficiency of the IgM antibodies identified in this study or of other naturally occurring antibodies can be demonstrated in sera of patients with other immune disorders. A study consisting of a broad survey of such sera is underway.

Table 2. Results of radioimmunoassay (*2*) of the reactivity of subfractions of sperm head proteins soluble in Triton X-100 (see Fig. 4) with IgM antibodies in normal human serum. ^{125}I-labeled goat antibody to human IgM was used as a probe. The total immunoreactivity of each pool was calculated as follows: $A_M \times M = R_t$, where A_M is the activity (in counts per minute) in 5 μg of protein, M is the amount of protein (in micrograms) in the pool, and R_t is the total reactivity. We have assumed a linear relation for the activity in 5 μg of protein, but even if this is not the case, the data show (i) that the immunoreactivity between the IgM antibodies and the fraction of sperm head proteins soluble in Triton X-100 is distributed similarly among the subsets of that fraction of proteins in the two normal human sera and (ii) that the major part of this reactivity (~90 percent) is in the proteins in pool 5. The fractions (see Fig. 4) were grouped as follows: pool 1, fractions 1 to 20; pool 2, fractions 21 to 28; pool 3, fractions 29 to 36; pool 4, fractions 37 to 49; pool 5, fractions 50 to 60; pool 6, fractions 61 to 68; and pool 7, fractions 69 to 100.

Fraction	M	Serum 1		Serum 2	
		A_M	R_t	A_M	R_t
Total		5261		5483	
Pool 1	15	17	50	14	42
2	1875	503	1.9×10^5	470	1.8×10^5
3	270	440	2.4×10^4	388	2.2×10^4
4	225	405	1.8×10^4	379	1.7×10^4
5	2115	5483	2.4×10^6	5568	2.4×10^6
6		14			
7		14			
	No protein	18			

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3. Sperm was collected by centrifugation and then washed three times in PBS (*p*H 7.2). A drop of sperm suspension, untreated or treated with Triton X-100 [T. C. Rodman *et al.*, *Gamete Res.* **8**, 129 (1983)], was placed on a slide, air-dired, and prepared (unfixed) for indirect immunofluorescence study with human serum.
4. The fluorescein isothiocyanate (FITC)–conjugated antibodies to human Ig's were selected to provide evidence of specific antibody recognition: $F(ab')_2$ goat antibody to human IgM (μ chain–specific); $F(ab')_2$ goat antibody to human IgG (Fc fragment–specific); and affinity-purified goat antibody to human serum IgA (α chain–specific). Use of $F(ab')_2$ fragments as the fluoresceinated probe eliminates the possibility of nonspecific reactivity of the probe with Fc receptors on sperm.
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6. A fraction of sperm heads, cleanly separated from tails, was treated with Triton X-100 without mechanical disruption of the heads (*1*). The proteins that were soluble in Triton X-100 were recovered in PBS (*p*H 7.2) as described [P. W. Holloway, *Anal. Biochem.* **53**, 304 (1973)] and subjected to electrophoresis on a sodium dodecyl sulfate gel. Blots of the separated proteins were tested with human sera and peroxidase-conjugated (goat) antibody to human IgM or IgG.
7. Total concentrations of IgM in six control sera (Table 1) were 55 to 103 mg per 100 ml and in four antibody-negative sera from patients at risk for AIDS (Table 1) were 39 to 99 mg per 100 ml. The IgM titer for the pediatric serum (Fig. 1B), which showed reactivity with sperm head proteins, was 55 mg per 100 ml; that for the AIDS serum (Fig. 1E), which showed no sperm head immunofluorescence, was 69 mg per 100 ml. Similarly, the IgM titer for the pediatric serum (Fig. 2, lane d) was 83 mg per 100 ml and that for the AIDS serum (Fig. 2, lane f and Fig. 3B) was 88 mg per 100 ml.
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Deregulation of Interleukin-2 Receptor Gene Expression in HTLV-I–Induced Adult T-Cell Leukemia

Abstract. *Infection of human T cells by human T-lymphotropic virus, type I (HTLV-I), a retrovirus, is uniformly associated with the constitutive expression of large numbers of cellular receptors for interleukin-2 (IL-2). Comparison with normal T cells shows that neither IL-2 receptor gene organization nor IL-2 receptor messenger RNA processing are altered in the leukemic cells. However, mitogenic stimuli activate IL-2 receptor gene expression in normal T cells, whereas these stimuli paradoxically inhibit IL-2 receptor gene transcription in HTLV-I–infected leukemic T cells.*

The type C retrovirus, human T-lymphotropic virus, type I (HTLV-I), has been identified as the etiologic agent in adult T-cell leukemia (ATL) (*1*). HTLV-I infection of human T cells is uniformly associated with expression of large numbers of cellular receptors for interleukin-2 (IL-2) (*2*, *3*). Together, IL-2 and its cellular receptor play an essential role in the control of normal T-cell growth (*4*). The relation of IL-2 receptor expression to HTLV-I infection is still unexplained. However, since most ATL cell lines do not transcribe IL-2 messenger RNA (mRNA) nor secrete IL-2, an autocrine growth model based on the continuous interaction of IL-2 with its receptor is unlikely (*5*). Furthermore, there is evidence that the IL-2 receptor is not the cellular receptor mediating entry of the HTLV-I virus (*6*). The finding that HTLV-I is not integrated at unique sites within the human genome argues against IL-2 receptor gene activation by adjacent insertion of HTLV-I promoter-enhancer sequences (*7*). We (*8*) and others (*9*) have recently isolated complementary DNA's (cDNA's) encoding the human IL-2 receptor. Using these IL-2 receptor cDNA probes, we have studied the deregulated expression of the IL-2 receptor gene in HTLV-I–infected T lymphocytes.

To investigate the possibility that IL-2 receptor expression reflects constitutive synthesis of IL-2 receptor mRNA, we used ^{32}P-labeled IL-2 receptor cDNA to analyze total cellular RNA from five ATL cell lines by Northern blotting. Each of these ATL lines constitutively expressed IL-2 receptor mRNA species similar in size to those present in mitogen-activated normal T cells (Fig. 1).

In an attempt to detect subtle differences between IL-2 receptor mRNA species from ATL cells and those from normal T cells, which might not have been evident in the Northern blotting analyses, we performed S1 nuclease protection studies with IL-2 receptor mRNA obtained from normal T cells and ATL cells (Fig. 2). As reported earlier (*8*), the formation of mature IL-2 receptor mRNA involves extensive post-transcriptional processing, including alternate splicing and the use of at least two, and probably three, separate polyadenylation [poly(A)] sites. In a first set of experiments, we used the Eco RI–Nae I cDNA fragment of pIL-2R3, corresponding to 910 base pairs (bp) at the 5′ end of the published sequence of pIL-2R3 (*8*). This fragment contains an internal 216-bp segment that may be removed by alternate splicing (*8*, *9*). Each of the ATL cell lines, like normal activated T cells, expressed both spliced and unspliced forms of IL-2 receptor mRNA's (Fig. 2). The spliced mRNA was detected in the S1 nuclease protection assay by identifying two fragments of sizes 549 bp and 155 bp, indicating the lack of protection within the 216-bp segment of the labeled Eco RI–Nae I fragment. The unspliced mRNA species is translated into an IL-2 binding receptor (*8*), but the function and the protein product encoded by the alternately spliced mRNA species are still undefined.

In a second series of S1 nuclease experiments, the 3′ Bgl I–Eco RI fragment of pIL-2R3 corresponding to base pairs

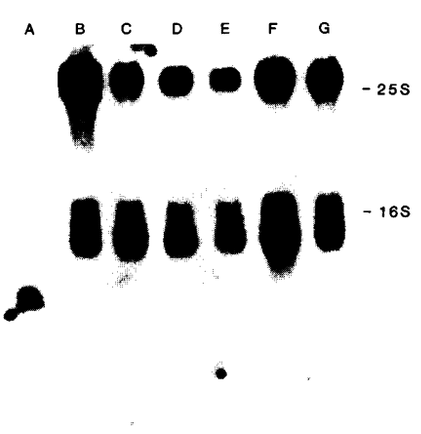

Fig. 1. Northern blot analysis of IL-2 receptor mRNA expression in normal T cells and ATL cell lines. Normal T cells were incubated in RPMI 1640 culture medium and stimulated for 18 hours with PHA (1 μg/ml) and PMA (50 ng/ml). Total cellular RNA (10 μg) from unstimulated T cells (A), T cells stimulated with PHA and PMA (B), and ATL cell lines HUT 102 (C), PL/P6 (D), C91/PL (E), MJ (F), and C5/MJ (G) were size-fractionated on formaldehyde-agarose gels, transferred to nitrocellulose filters, and hybridized to pIL2R2 and pIL2R4 cDNA probes (*8*) labeled with ^{32}P by nick translation. The origin and cell surface phenotype of these cell ATL lines have been described (*3*).