duce the same population density scaling inside and outside both marine preserves. To explore scaling patterns in other intertidal communities with a different species composition might prove the generality of this relation and help explain the underlying mechanisms.

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8. The marine preserve at Las Cruces (33°30'S, 71°38'W) encloses a 500-m stretch of exposed rocky shore, from which man has been excluded for more than 6 years. The marine preserve at Montemar (32°57'S, 71°33'W), presents similar characteristics, but man has been excluded for more than 11 years [J. C. Castilla, *Ambiente Desarollo (Chile)* **2**, 53 (1986)].
9. After human exclusion at Las Cruces (*8*), increased densities of the predatory gastropod *Concholepas concholepas* produced drastic changes in local species diversity. Density and size of several invertebrate and algae species also changed, and man-induced cascade effects and indirect interactions have been apparent when comparing inside and outside communities in both marine preserves. The main changes at Las Cruces are discussed by J. C. Castilla and L. R. Durán [*Oikos* **45**, 391 (1985)]; D. Oliva and J. C. Castilla [*Publ. Stazione Zool. Napoli Mar. Ecol.* 7, 201 (1986)]; J. C. Castilla (*8*); L. R. Durán, J. C. Castilla, and D. Oliva [*Environ. Conserv.* **14**, 143 (1987)]; J. C. Castilla [*Arch. Biol. Med. Exp.* **20**, 146 (1987)]; and L. R. Durán and J. C. Castilla (*10*). Similar changes occurred after exclusion of humans from the marine preserve of Montemar [J. C. Castilla (*8*); *Informe Unesco Cienc. Mar.* **47**, 115 (1988)].
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11. Transects were placed haphazardly on rocky platforms with 20° to 45° of slope. Along them, cover of sessile animals was measured with a 1-m^2 quadrat (196 regular intersection points), placed continuously from the lowest to the highest tidal levels. Density of sessile species was obtained by counting individuals in 100-cm^2 quadrats and multiplying by the corresponding cover. Densities of periwinkles and limpets were evaluated with 0.10-m^2 quadrat; all other organisms were evaluated with 1-m^2 quadrat. Data entered in the analysis are averages of samples conducted in March and April of 1986 for both preserves, December 1988 and October 1989 for Las Cruces, and February and September 1989 for Montemar. Additional data for species inside holdfasts of macroalgae and mussel beds were obtained from other sources [J. Cancino and B. Santelices, *Rev. Chil. Hist. Nat. (Chile)* **57**, 23 (1984); M. Muñoz and B. Santelices, *Mar. Ecol. Prog. Ser.* **54**, 277 (1989)]. Density ranged from 0.52 to 30,071 (individuals m^{-2}) and body size from 0.0003 to 173.46 g.
12. Representative measures of length (millimeters) of at least 150 individuals of each species were taken in randomly selected quadrats along the transects.
13. Body size (weight) of species was obtained from logarithmic regressions of modal length (measured in millimeters) versus weight (grams) of individuals (including shells). Regressions were based on 30 to 120 individuals collected in November 1988 and March 1989.
14. Comparison of slopes by t tests (d.f. = 46): $P > 0.05$.
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16. Analysis of covariance comparing regression lines inside and outside marine preserves. Las Cruces: intercepts $P = 0.72$ (d.f. = 1,51); slope $P = 0.61$ (d.f. = 1,51); Montemar: intercepts $P = 0.73$ (d.f. = 1,49); slopes $P = 0.70$ (d.f. = 1,49).
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18. Because individual basal metabolic requirements (R) scale with size (M) by the 0.75 power [M. Kleiber, *The Fire of Life* (Kriger, New York, 1975)] and population density (D) scales with body size by the −0.75 power, the energy used by a local population of a species ($D \times R$) scales with body size as M^0.
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Detection of a Human Intracisternal A-Type Retroviral Particle Antigenically Related to HIV

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Sjögren's syndrome is an autoimmune disease that is characterized by dryness of the mouth and eyes. The loss of salivary and lacrimal gland function is accompanied by lymphocytic infiltration. Because similar symptoms and glandular pathology are observed in certain persons infected with human immunodeficiency virus (HIV), a search was initiated for a possible retroviral etiology in this syndrome. A human intracisternal A-type retroviral particle that is antigenically related to HIV was detected in lymphoblastoid cells exposed to homogenates of salivary tissue from patients with Sjögren's syndrome. Comparison of this retroviral particle to HIV indicates that they are distinguishable by several ultrastructural, physical, and enzymatic criteria.

SJÖGREN'S SYNDROME (SS) IS AMONG several autoimmune diseases that overlap clinically with diseases induced by HIV (*1, 2*). The characteristic symptom of SS is dryness of the mouth and eyes, which is also sometimes observed as a manifestation of HIV infection (*2*). The dryness in both SS and HIV disease is due to loss of salivary and lacrimal gland function and is accompanied by lymphocytic infiltration of these glands. An additional link between SS and HIV disease is our observation that approximately 30% of primary SS patients produce serum antibodies that react with the major capsid protein of HIV (CA, p24/25) (*3*). In some SS patients, reactivity to another Gag protein, p17 (MA), was also observed. Similar percentages of patients with systemic lupus erythematosus (SLE), scleroderma, and juvenile rheumatoid arthritis (JRA) also produce HIV Gag-reactive serum antibodies (*3, 4*). Lower percentages (1 to 4%) of healthy individuals or individuals with other chronic diseases produce antibodies cross-reactive to HIV proteins. These observations suggest the possibility of a retroviral etiology in SS and perhaps in other autoimmune diseases.

The pathology of SS is more localized than other autoimmune diseases. Therefore, we attempted to culture an infectious agent from SS patients. Salivary gland tissue was collected by lip biopsies of six persons with SS, homogenized in a tissue grinder, and the crude tissue homogenates were added to cultures of the RH9 subclone of HUT 78, a T-lymphoblastoid cell line (*5*). After 6

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weeks, detergent lysates of cells exposed to one salivary gland biopsy (RH9/MC) became positive for HIV-related Gag antigens as shown by an antigen-capture enzyme-linked immunoassay (EIA) for HIV proteins (Abbott Laboratories). Cell-free supernatants from RH9/MC cells did not contain detectable levels of HIV-related antigen. Lysates of cells exposed to homogenates of a second biopsy (RH9/HD) became positive for expression of HIV-related antigenic reactivity after 12 weeks in culture. Concentrated extracts of uninfected RH9 cells did not react in the EIA. Reactivity of RH9/MC and RH9/HD cells was blocked by addition of recombinant HIV CA produced in a baculovirus:insect cell system (Micro-Gene-Sys). Cells cultured with the four other biopsies from SS patients remained negative for HIV-related antigen as seen by EIA for more than 24 weeks. Cytopathic effects were not observed during the 6- to 12-week interval when the RH9/MC and RH9/HD became positive for HIV-related antigen; however, the doubling time of these cells (12 to 15 hours) decreased from that of uninfected RH9 cells (18 to 24 hours). We have confirmed by karyotype analysis and immunological analysis of cell surface markers that these cells are exclusively of human origin. Primers from conserved regions of HIV *gag*, *pol*, and *env* genes in the polymerase chain reaction run under conditions of low stringency did not amplify nucleic acid sequences from these cells, indicating that they were not infected with a defective HIV (*6*).

To determine whether expression of the HIV-related antigenic reactivity of cells exposed to SS patient salivary gland homogenates was due to production of a viral particle, the cells were examined by transmission electron microscopy. HIV, a lentivirus, matures principally at the plasma membrane of infected T-lymphoblastoid cells. Despite the fact that the cells were reacting to HIV-specific antibodies, the RH9/MC cells did not produce viral particles that matured at the plasma membrane. This explains the lack of HIV-related antigen in culture supernatants from these cells. However, we did observe retroviral particles contained within intracytoplasmic vacuoles in some cells of this culture (Fig. 1A). The intracisternal particles consisted of two electron-dense concentric rings having a "doughnut-shaped" appearance. One or two particles were generally found per thin section through a region of the cell containing cytoplasmic vacuoles. Particles with this distinctive morphology were also found in RH9/HD cells, but were not found in RH9 cells infected with HIV (Fig. 1B) or in uninfected RH9 cells, despite an extensive search throughout various levels of the cells. The particles from the RH9/MC and RH9/HD cells are ultrastructurally similar to intracisternal A-type particles, which have been described in a variety of normal and transformed cells from other species (*7, 8*), and so we will refer to them as human intracisternal A-type particles (hIAP).

To physically characterize the particles produced by the RH9/MC cells, we used protocols developed for murine A-type retroviruses (*9*). A peak of HIV-related antigen from RH9/MC cell lysates was detected by antigen-capture EIA in a 33 to 68% sucrose gradient, the last step in this procedure, at a density of ~1.22 g/ml (Fig. 2A, fractions 9 to 11). This density is similar to that of A-type particles from other species (*8, 9*). Particles with typical A-type morphology were observed by phosphotungstic acid staining in these fractions, but not in fractions from other parts of the gradient (*10*). Furthermore, antibodies in 16 of 18 SS or SLE patient sera reacted with one or more proteins of relative molecular mass (M_r) of 45,000, 61,000, and 71,000 localized to these gradient fractions, and a rabbit serum monospecific for HIV CA reacted with the M_r 61,000 and 70,000 proteins (*4*). As expected, HIV virions sedimented in a parallel gradient at a density of 1.14 to 1.16 g/ml (Fig. 2B, fractions 4 to 6) (*11*).

One defining characteristic of retroviruses, including A-type retroviral particles, is the presence of an RNA-dependent DNA polymerase (reverse transcriptase, RT) in the viral particle (*12–15*). Reverse transcriptases of various retroviruses can often

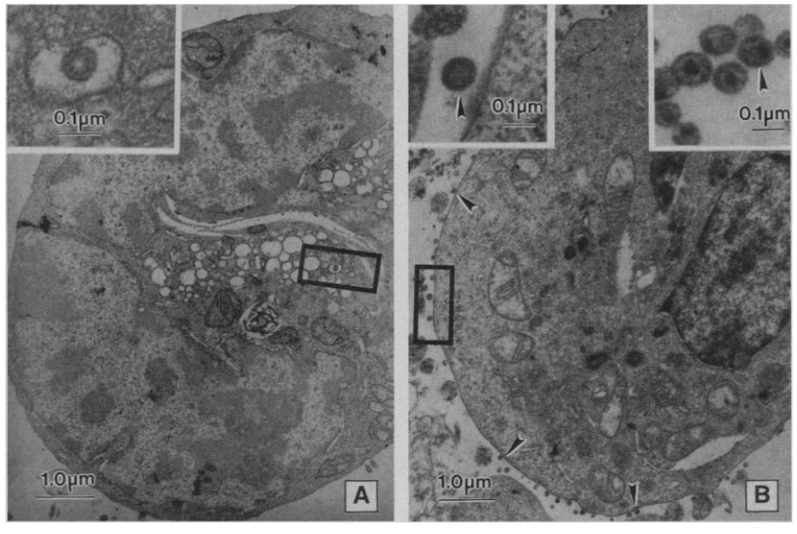

Fig. 1. Ultrastructure of intracisternal A-type retrovirus particles in RH9 cells exposed to salivary gland extracts from SS patients. RH9/MC were examined by transmission electron microscopy (*21*). (**A**) RH9/MC containing a typical intracisternal A-type retrovirus (in the rectangle). Inset: Higher power micrograph of the hIAP enclosed by the rectangle in (A). (**B**) RH9 cells persistently infected with the LA1 strain of HIV-1 prepared by same procedures as hIAP-infected cells. Arrowheads indicate HIV at various stages of budding from the plasma membrane. Left inset: Higher power micrograph of the immature HIV virion enclosed by the rectangle in (B) (arrowhead). Right inset: HIV virion including a particle with a typical lentivirus core (arrowhead).

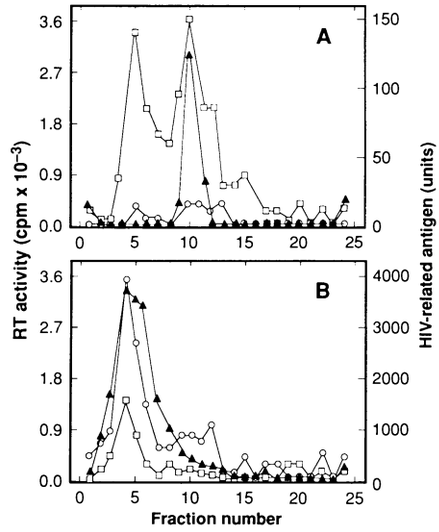

Fig. 2. RH9 cells exposed to salivary gland extracts produce particles antigenically related to HIV with the hydrodynamic properties of an intracisternal A-type particle. Lysates of RH9 cells exposed to salivary gland extracts of patient MC (RH9/MC) were subjected to a procedure previously used for purification of intracisternal A-type retroviral particles from other species (*9*). Fractions from the 33 to 68% linear sucrose gradient, the last step in the procedure, were tested for the presence of RT activity with 0.1 mM Mn^{2+} (□) or 5.0 mM Mg^{2+} (○) and for the presence of HIV-related antigens by an antigen-capture EIA (▲). Values are the average of two to four determinations. Portions from each fraction were also tested for refractive index to determine density. (**A**) Gradient analysis of hIAP. (**B**) Concentrated supernatants from RH9 cells persistently infected with HIV-1 (strain LA1) subjected to isopycnic banding.

be distinguished by substrate or ionic preferences (*13*). Therefore, the abilities of detergent-disrupted preparations of hIAP and HIV to synthesize DNA were compared at various concentrations of Mg^{2+} and Mn^{2+}, with polyadenylate [poly(A)] as a synthetic template and oligo(dT) as a primer. The highest RT activities associated with hIAP preparations were obtained when Mn^{2+} was used as the divalent cation (Fig. 3A).[3] The hIAP-associated RT activity was higher with Mn^{2+} than with Mg^{2+} (at or near the optimum concentrations) in each of six independent experiments. In confirmation of previous studies, HIV RT activity was higher with Mg^{2+} than with Mn^{2+} (Fig. 3B) (*14*). These results indicated that the hIAP-associated RT differed from that of HIV. In additional studies, we observed that the hIAP-associated RT activity was higher with RNA [poly(A)] than with DNA [poly(dA)] as template (*16*). Furthermore, the hIAP preparation could not effectively synthesize DNA in a reaction mixture containing only oligo(dT) (*16*). The preparation did not contain sufficient levels of a DNA-dependent DNA polymerase nor a deoxyribonucleotidyl (terminal) transferase to account for the observed DNA polymerase activity.

Fractions from the sucrose gradients used to compare the hydrodynamic properties of HIV and the hIAP (Fig. 2) were also assayed for Mg^{2+}- and Mn^{2+}-dependent RT activities. As expected, the major HIV peak corresponded to the peak of Mg^{2+}-dependent RT activity (Fig. 2B). In contrast, the major peak of HIV-related antigen from RH9/MC (1.22 g/ml) corresponded to a major peak of Mn^{2+}-dependent RT activity (Fig. 2A) and thus could be distinguished from HIV by differences in both hydrodynamic mobility and divalent cation preference. An additional peak of RT activity not associated with HIV-related antigen was also detectable in lysates of RH9/MC cells. It could be distinguished from the hIAP-associated RT by sedimentation properties (Fig. 2A, fraction 5 versus fraction 10), and an over 30-fold higher RT activity with Mn^{2+} than with Mg^{2+} (*16*). This is probably an endogenous RT activity, as is commonly observed in uninfected cells (*15*). When detected in lysates of uninfected RH9 cells, this cellular RT migrated as a single peak at 1.17 to 1.19 g/ml and was not associated with HIV-related antigen.

The isolation of a retroviral particle from cells exposed to salivary gland homogenates of patients with SS raises the possibility that this agent is the antigenic stimulus for production of Gag-reactive antibodies in sera from autoimmune patients observed by ourselves and others (*3, 17*). However, the

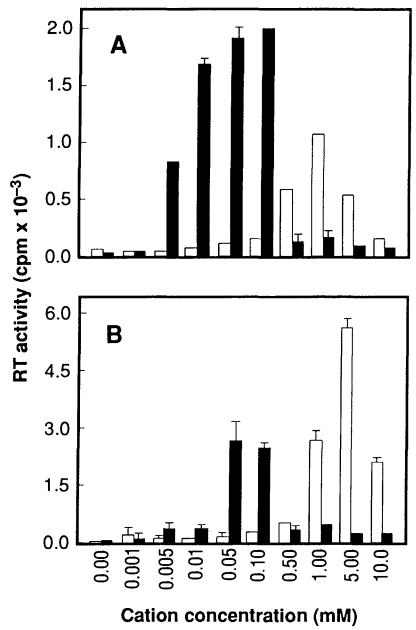

Fig. 3. Divalent cation preference of the RT activities associated with hIAP and HIV. Reverse transcriptase reaction mixtures containing poly(A):oligo(dT) as template and primer and containing the indicated amount of Mg^{2+} (white bars) or Mn^{2+} (black bars) were incubated for 60 min. The amount of radioactivity from [^{3}H]thymidine triphosphate that became acid precipitable was determined. Values are averages of duplicate determinations plus or minus the standard error of the mean. Differences between the HIV- and hIAP-associated RT activities in this range of Mn^{2+} and Mg^{2+} concentrations were significant at $P > 0.0001$ as determined by analysis of variance (ANOVA0. **A**) hIAP. (**B**) HIV-1 (strain LA1).

studies presented here obviously do not provide proof that the agent we have identified is involved in the etiology of SS or other autoimmune diseases. On the other hand, an association between retrovirus infections and autoimmune phenomena has long been suspected (*17, 18*). Transgenic mice expressing the *tax* gene of human T cell lymphotrophic virus (HTLV-I) have recently been shown to develop exocrinopathy resembling SS (*19*). Other recent studies have linked retrovirus-like nucleic acids to SLE and Graves' disease (*20*). Determination of the relatedness of the hIAP to HIV or other retroviruses must await cloning and sequencing of the genetic material responsible for producing the hIAP. Such information may prove valuable in elucidating the origins of human retroviruses and understanding the mechanisms by which they induce immune dysfunctions.

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