reversion of the constructs to an infectious form or for horizontal spread of HFV (*13*). It is unlikely that expression of the HFV constructs depends on the cell cycle because neurons are irreversibly postmitotic in adult animals. These observations indicate that additional, unknown cellular factors may be required for high levels of HFV LTR–directed transcription.

Because expression of HFV was predominantly seen in anatomical structures that will eventually degenerate, it is likely that the gene products of HFV are directly responsible for the observed lesions. However, we are unable to identify conclusively which proteins of HFV are responsible for neurotoxicity. Nerve cell degeneration was present in animals carrying the pΔgpe DNA, which essentially contains only the *bel* reading frames. A possible candidate for neurotoxicity is the *bel-1* gene product, which is functionally comparable to the retroviral transactivating factors, HIV *tat* and HTLV-I *tax* (*5–7*). However, transgenic mice carrying LTR *tat* and LTR *tax* develop Kaposi sarcomas and peripheral neurogenic and mesenchymal tumors, respectively (*10*), but no degenerative encephalopathy. These differences may relate to the tissue-specific expression pattern caused by the HFV cis regulatory elements or to different cellular and molecular targets of the *bel-1* protein.

The pathologies observed in HFV mice are reminiscent of human retroviral disease of the CNS: neuronal loss and white matter damage are often present in HIV-associated encephalopathy (*14*) and vacuolar myelopathy (*15*) and in HTLV-I–induced disease of the CNS (*16*). The mechanisms of neurotoxicity operating in these conditions may be related to those leading to neurological damage in HFV transgenic mice. In fact, one of the original isolates of HFV was obtained from the brain of a patient with a neurodegenerative disease with features reminiscent of the lesions in the HFV mice (*2*).

Although a specific pathology has not been unequivocally associated with HFV infection, and foamy viruses generally have been regarded as benign (*4*), detection of HFV in human diseases might be problematic if virulence is restricted to immunocompromised hosts or to abortive forms of infection (*17*). The findings described here prompt a critical reappraisal of the potential role of HFV as a human pathogen. It will be crucial to search for evidence of HFV infection, or superinfection, in neurodegenerative and myopathic disorders of unknown etiology and neurological syndromes in immunodeficient patients.

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Identification of Profilin as a Novel Pollen Allergen; IgE Autoreactivity in Sensitized Individuals

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A complementary DNA encoding a pollen allergen from white birch (*Betula verrucosa*) that was isolated from a pollen complementary DNA library with serum immunoglobulin E from a birch pollen–allergic individual revealed significant sequence homology to profilins. The recombinant protein showed high affinity to poly-L-proline. Immunoglobulin E antibodies from allergic individuals bound to natural and recombinant birch profilin and also to human profilin. In addition, birch and human profilin induced histamine release from blood basophils of profilin-allergic individuals, but not of individuals sensitized to other plant allergens. The structural similarity of conserved proteins might therefore be responsible for maintaining immunoglobulin E antibody titers in type I allergy.

ALLERGIES OF THE IMMEDIATE TYPE are a major health problem in industrialized countries, where up to 15% of the population suffers from type I allergic symptoms (rhinitis, conjunctivitis, and bronchial asthma). An understanding of the complex pathogenetic mechanisms leading to allergy requires information about the structure and function of allergenic proteins. Comparison of the sequences of cloned allergens with those of known proteins has already contributed to the characterization of some allergenic proteins. The major birch (*Betula verrucosa*) pollen allergen Bet v I (*1*) and the white-faced hornet venom allergen antigen 5 Dol m V (*2*) were shown to be highly homologous to pathogenesis-related plant proteins. The major house dust mite allergen Der p I is homologous to proteases-

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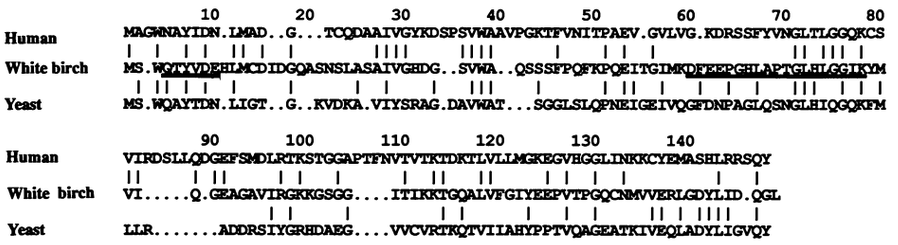

Fig. 1. Amino acid (*23*) sequences of human (*15*) and yeast (*12*) profilins are compared with the birch profilin sequence. Identical amino acid residues are marked by vertical lines. Gaps are indicated by dots. Amino acid residues that were determined by sequencing tryptic fragments of natural birch pollen profilin are underlined. Sequence identity between birch and human profilins is 34% and between birch and yeast profilins is 32%, whereas sequence identity with slime mold (*10*) and amoeba (*11*) profilins is 43 and 38%, respectively. Numbers above the sequence indicate the residue number, including gaps, starting at initiator methionine.

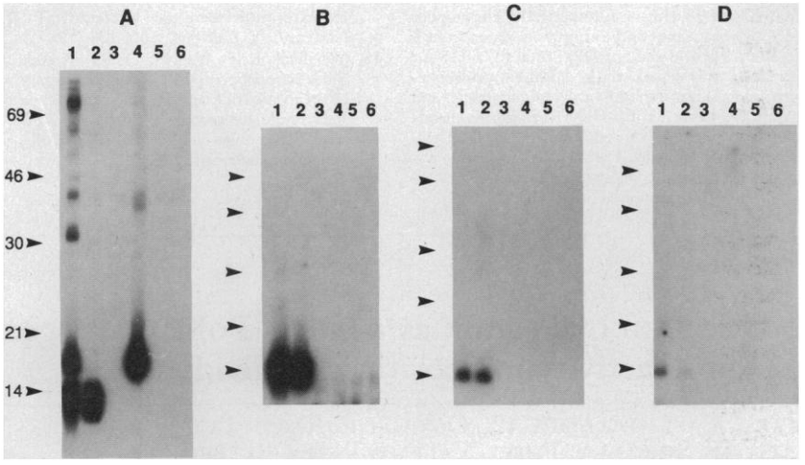

Fig. 2. Autoradiography of IgE immunoblots; molecular size standards (in kilodaltons) are indicated at the left side. Arrows indicate the relative positions of the same markers on the other gels. (**A**) Total proteins of a birch pollen extract were separated with 12% denaturing polyacrylamide gel electrophoresis and blotted onto nitrocellulose that was subsequently cut into strips. Strips were incubated with sera of allergic individuals diluted one to ten in buffer A [50 mM sodium phosphate (pH 7.5), 0.5% Tween 20, 0.5% bovine serum albumin, and 0.05% sodium azide], and bound IgE was detected with iodinated rabbit antibody to human IgE (Pharmacia, Uppsala, Sweden). (**B**) Birch profilin was purified by affinity chromatography on poly-L-proline–Sepharose (*21*), electrophoresed in a denaturing polyacrylamide gel, and blotted onto nitrocellulose. (**C**) The cDNA coding for birch profilin was expressed in pKK223-3 (*20*), the recombinant protein was purified (*21*), subjected to electrophoresis, and blotted onto nitrocellulose. (**D**) Sera IgE that had bound to natural and recombinant profilin (patients 1 and 2) also reacted with human profilin purified from platelets. The purification procedure was based on the known affinity of profilins to poly-L-proline (*21*). Lane 1, the serum of an individual allergic to most birch pollen allergens, in particular to the major birch pollen allergen Bet v I (*1*) at 17 kD, to a 14-kD protein, and to a series of proteins in the range between 30 to 90 kD. Lane 2, serum of an individual who reacts exclusively to the 14-kD protein. Lane 3, a pool of sera from five nonallergic individuals. Lane 4, serum that bound to a 17-kD protein (Bet v I). Lane 5, serum from an individual allergic to house dust mites, but without IgE reactivity to birch. Lane 6, buffer control without added serum.

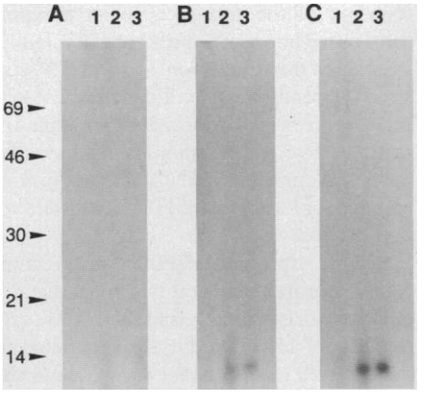

Fig. 3. Competitive inhibition of IgE binding. Human profilin purified as described (*21*) was subjected to electrophoresis and blotted onto nitrocellulose. Strips of nitrocellulose were incubated with dilutions of serum 1 (Fig. 2) that had been incubated in the fluid phase with (lane 1) 10 μg of purified recombinant profilin, with (lane 2) control proteins from *Escherichia coli* JM 105 transformed with the plasmid pKK223-3 without the profilin cDNA insert, or with (lane 3) buffer A. (**A**) No added serum, (**B**) 1 to 20 dilution of serum, and (**C**) 1 to 10 dilution of serum. Bound IgE was detected as described in Fig. 2. Molecular size markers are shown at left in kilodaltons.

(*3*) and has protease activity itself (*4*).

We cloned and sequenced cDNAs encoding immunoglobulin E (IgE)–binding birch pollen proteins distinct from Bet v I. One of these allergens was identified as birch profilin by sequence homology and affinity to poly-L-proline. The protein is recognized by IgE antibodies in about 10% of those individuals allergic to birch pollen (*5*) and seems to be an intermediate or major allergen in individuals allergic to pollens of grasses and weeds (*6*). Immunoglobulin E antibodies from birch profilin–allergic individuals cross-reacted with human profilin, and histamine release from peripheral blood basophils could be induced with the human protein. This IgE reactivity to a self antigen suggests a mechanism that could maintain a high titer of IgE in individuals allergic to profilins.

A birch pollen cDNA library was constructed in λ gt11 and screened for IgE-binding proteins with the use of serum IgE from an allergic individual selected according to case history and results from a RAST (radioallergosorbent test) and an IgE immunoblot pattern with pollen extracts (*5*). A partial 5′-deleted cDNA clone was isolated. We used oligodeoxynucleotides complementary to the coding region to obtain two independent complete cDNA clones by plaque hybridization (*7*); these clones were then sequenced (*8, 9*). A cross-hybridizing mRNA of approximately 800 nucleotides was detected in birch, alder, and hazel pollen. The deduced amino acid sequence of these cDNA clones (Fig. 1) was homologous to profilins from slime mold (*Physarum polycephalum*) (*10*), amoeba (*Acanthamoeba castellanii*) (*11*), yeast (*Saccharomyces cerevisiae*) (*12*), mouse (*13*), calf (*14*), and man (*15*).

Profilins regulate actin polymerization (*16*) and participate in signal transduction by way of the phosphoinositide signaling pathway (*17*). In particular, they function as actin-sequestering proteins in the acrosomal reaction of echinoderm sperm (*18*). Most of the clusters of high sequence homology between the different profilins are located either toward the NH_2-terminus or the COOH-terminus of the molecule. Both regions are presumed to participate in the interaction with actin (*19*). We observed large amounts of profilin in pollens of trees [birch, alder (*Alnus glutinosa*), and hazel (*Corylus avellana*)], grasses [timothy grass (*Phleum pratense*) and rye (*Secale cereale*)], and weeds [mugwort (*Artemisia vulgaris*)] (*6*). This may suggest a function for pollen profilins in plant fertilization.

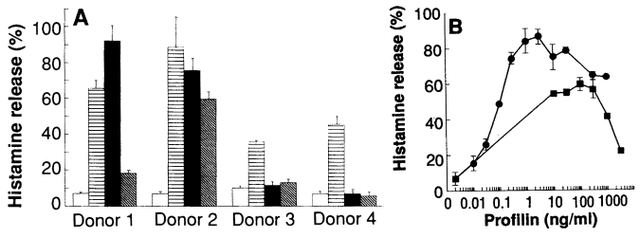

Fig. 4. Histamine release from basophils prepared from peripheral blood (*22, 24*). (**A**) Basophils from individuals allergic to birch profilin (donors 1 and 2) and from two individuals allergic to pollen proteins other than profilin (tested by IgE immunoblots) (donors 3 and 4) were tested for histamine release after sensitization with birch profilin (solid bars) or human profilin (hatched bars) and on stimulation with monoclonal antibody E124-2-8 to human IgE (*24*) (wide, horizontal bars). Open bars represent nonspecific release observed with the addition of buffer. Results shown are after stimulation with an optimal concentration of antigen that resulted in the maximum percentage of histamine release. Individuals had been characterized by case history and results from a RAST and IgE immunoblots (*5*). (**B**) Dose dependency of histamine release. Granulocytes from donor 2 were exposed to various concentrations of birch profilin (●) and human profilin (■). Histamine release is expressed as a percentage of total histamine. The values are means of triplicate determinations, and the error bars indicate the standard deviation.

The complete cDNA that codes for the putative birch profilin was expressed in pKK223-3 (*20*). The recombinant protein, as well as a birch pollen protein of the same size and IgE-affinity, could be purified by affinity chromatography on poly-L-proline–Sepharose (Sigma), a method specific for profilins (*21*). The sequence of two tryptic fragments from the purified natural birch profilin exactly matched a portion of the amino acid sequence deduced from the cDNA sequence (Fig. 1). Profilins were also purified from pollens of grasses (*P. pratense* and *S. cereale*) and weeds (*A. vulgaris*) and identified as relevant allergens by IgE immunoblot and IgE immunoblot inhibition experiments with 60 individuals (*6*). Because these profilins derived from pollens of plants belonging to distantly related species share common IgE-binding epitopes, the profilins can be regarded as a group of "panallergens."

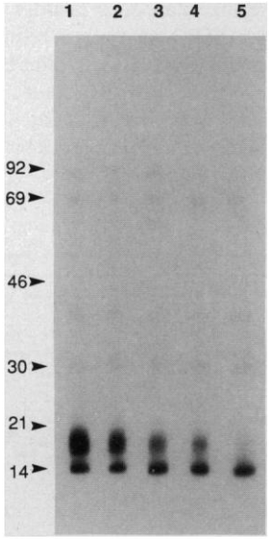

Fig. 5. Immunoglobulin E immunoblot of birch pollen proteins. Serum samples from a birch pollen–allergic individual that had been collected throughout one year. Lane 1, end of February; lane 2, May; lane 3, June; lane 4, October; and lane 5, January. The birch pollen season extended from the end of February to the end of May in the year in which the samples were obtained. We used sera to detect blotted birch pollen allergens, and bound IgE was detected as described in Fig. 2. Molecular size standards are indicated at left in kilodaltons.

Although computer-aided predictions of secondary structure did not clearly indicate structural homology between birch and human profilins, we analyzed sera from individuals with high IgE titers to natural and recombinant birch profilin for IgE reactivity to human profilin. These sera were tested for their capacity to bind human profilin on immunoblots (Fig. 2). Sera with IgE to purified natural and recombinant birch profilin (Fig. 2, B and C) also recognized human profilin with their IgE (Fig. 2D), but these antibodies were of lower affinity. Binding of human IgE to human profilin could be blocked with recombinant birch profilin that was added in the fluid phase as a native protein (Fig. 3), which indicates that there are common epitopes. These epitopes might be conformational rather than sequential.

On the basis of these data, we investigated whether the autoreactivity of this IgE might affect the in vivo allergy. We used histamine-release tests (*22*) to determine whether the cross-reactive antibodies were able to elicit allergic effector mechanisms. Basophils from three individuals allergic to birch profilin, from two individuals allergic to pollen proteins other than profilin, and from five nonallergic donors were tested for histamine release in response to birch and human profilins. Response to human profilin was observed only with individuals who had high IgE antibody titers to the birch pollen profilin (Fig. 4). It is therefore possible that at least some of the allergic symptoms caused primarily by sensitization of mast cells with pollen profilin may be aggravated or prolonged by human profilin.

Although serum IgE is relatively short-lived, individuals allergic to profilin had high IgE titers, even at times outside the pollen season, perhaps sustained by endogenous profilin acting as a booster. Throughout one year, we measured the reactivity of IgE to birch profilin and to the major birch pollen allergen Bet v I (*1*) that does not have a known human homolog. Serum samples were collected from an individual allergic to both allergens. The intensity of IgE reactivity to birch profilin remained constant through the year, even outside the pollen season, whereas the IgE reactivity to Bet v I decreased outside the pollen season (Fig. 5). We have described an example of a specific IgE antibody that is reactive to a human protein. Whether the cause of this humoral autoimmunity is formation of autoantibody to the human profilin or cross-reactivity of antibody to the birch profilin with the human profilins (molecular mimicry) remains to be answered.

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Protein Kinase Activity Closely Associated with a Reconstituted Calcium-Activated Potassium Channel

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Modulation of the activity of potassium and other ion channels is an essential feature of nervous system function. The open probability of a large conductance Ca^{2+}-activated K^+ channel from rat brain, incorporated into planar lipid bilayers, is increased by the addition of adenosine triphosphate (ATP) to the cytoplasmic side of the channel. This modulation takes place without the addition of protein kinase, requires Mg^{2+}, and is mimicked by an ATP analog that serves as a substrate for protein kinases but not by a nonhydrolyzable ATP analog. Addition of protein phosphatase 1 reverses the modulation by MgATP. Thus, there may be an endogenous protein kinase activity firmly associated with this K^+ channel. Some ion channels may exist in a complex that contains regulatory protein kinases and phosphatases.

MODULATION OF NEURONAL ACTIVITY underlies many changes in behavior. Such modulation often results from changes in the activity of membrane ion channels. Ca^{2+}-activated K^+ channels, one subclass of the diverse and widespread family of K^+ channels, are intriguing in this regard because they provide a link between second messenger systems and the membrane potential. Some members of this channel subclass are targets for protein phosphorylation, a well-investigated modulatory mechanism for ion channels (*1*). Adenosine 3′,5′-monophosphate (cAMP)–dependent protein kinase (PKA) (*2*) and protein kinase C (PKC) (*3*) both change the activity of Ca^{2+}-activated K^+ channels in a number of tissues.

We have described the properties of several different types of Ca^{2+}-activated K^+ channels from rat brain incorporated into planar lipid bilayers (*4*). Addition of the purified catalytic subunits of PKA and protein phosphatase 2A (PP-2A) can modulate the gating of two different types of large-conductance Ca^{2+}-activated K^+ channels from brain (*5*). These two channel types have the same unitary conductance (240 pS in symmetrical 150 mM K^+) but differ in their gating kinetics, Ca^{2+} sensitivity, pharmacology, and response to phosphorylation by PKA. We have now investigated the modulation by MgATP of the slower gating (*4–6*) of these large-conductance Ca^{2+}-activated K^+ channels.

We recorded single-channel activity for such a channel under conditions in which the open probability was low (Fig. 1A). When 0.5 mM ATP was added to the cytoplasmic side of the channel, the open probability increased substantially (Fig. 1B). With some channels, as little as 50 μM ATP was enough to produce similar increases in open probability. In 25 separate experiments, 16 channels exhibited a large increase in open probability after the addition of ATP, whereas in 9 experiments ATP was without effect. From among the 16 ATP-modulated channels, we chose for quantitative analysis 6 representative channels in which the starting open probabilities were similar. ATP increased the maximal open probability of these channels from 0.058 ± 0.013 (mean ± SEM) to 0.449 ± 0.161. ATP added to the extracellular side of the bilayer did not alter channel activity.

We next examined the time dependence of the open probability changes induced by ATP (Fig. 2A). In 16 experiments the lag time between the addition of ATP and the increase in open probability varied from channel to channel and ranged from less than 20 s to as long as 3 min. The open probability of the channel after the addition of ATP was not constant but oscillated between lower and higher open probability values (Fig. 2, A and B). In the absence of ATP, we never observed these rapid, large changes in channel open probability (Fig. 2C).

Magnesium was required for the modulation by ATP. When buffer containing 1 mM Mg^{2+} was replaced with Mg^{2+}-free solution containing 20 μM EDTA, 1 mM ATP was no longer able to increase channel activity (n = 5). To examine whether ATP hydrolysis was necessary for the modulation, we tested analogs of ATP. In eight independent experiments, we observed no increase in the open probability in the presence of up to 1 mM of the nonhydrolyzable ATP analog, AMPPNP (adenylylimidodiphosphate). In contrast to AMPPNP, ATPγS [adenosine-5′-*O*-(3-thiotriphosphate)], a hydrolyzable ATP analog that can substitute for ATP in most kinase reactions (*7*), increased the activity of the Ca^{2+}-activated K^+ channel in 12 of 14 experiments (Fig. 3). Quantitative analysis of four representative experiments, with similar initial open probabilities, revealed a greater than tenfold increase in channel open probability in response to ATPγS (from 0.060 ± 0.009 to 0.667 ± 0.083). ATPγS generally took longer to modulate the channel than ATP, consistent with reports that it is used at slower rates in phosphotransferase reactions (*7*). Furthermore, ATPγS induced a more stable, high open probability state (Fig. 3) than did ATP (Fig. 2, A and B).

These results with ATP analogs demonstrated that ATP hydrolysis is required for channel modulation. This is different from the ATP-sensitive channels found in a variety of tissues, in which ATP functions as a ligand to alter channel properties by binding

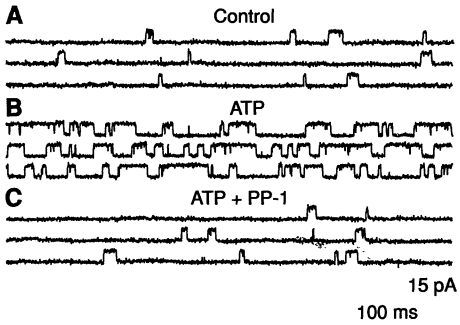

Fig. 1. Activation of a large-conductance Ca^{2+}-activated K^+ channel in lipid bilayers by MgATP and reversal by PP-1. Voltage across the bilayer was clamped at +10 mV, and the reversal potential for K^+ was set to −28 mV. For all traces, channel openings are shown as upward deflections from the closed-level current. (**A**) Representative control openings of the channel before the addition of ATP in three continuous traces. (**B**) Traces 3 min after the addition of 0.5 mM ATP (Calbiochem) to the cytoplasmic side. (**C**) The effect of MgATP is reversed by the addition of 30 nM PP-1 in the continued presence of MgATP.

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