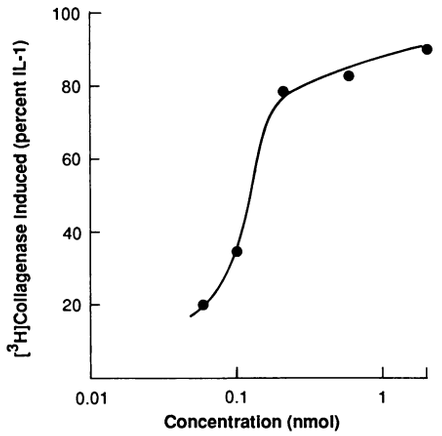

Fig. 4. Concentration dependence of rabbit proteins on collagenase induction of human skin fibroblasts. Various concentrations of purified 12-kD and 14-kD proteins, *p*I 8, were tested for their ability to induce collagenase (Fig. 2). IL-1 (60 ng/ml) represents a positive control.

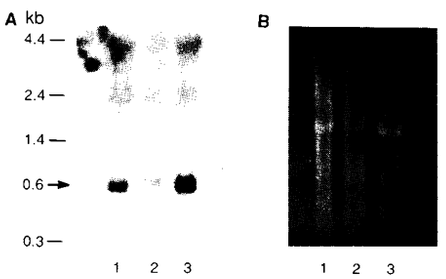

Fig. 5. RNA blot analysis of rabbit mRNA hybridizing with a human SAA probe. Whole-cell RNA (25 μg) from untreated control or rabbit fibroblasts stimulated with PMA for 48 hours was separated on a 1% agarose gel containing 2.2*M* formaldeyhyde (*16*). After electrophoresis in phosphate buffer (*16*), the gel was transferred to a GeneScreen plus filter (Du Pont, Biotechnology Systems) and hybridized with a [^{32}P]deoxycytidine triphosphate–oligolabeled cDNA probe for human SAA (*5*) under stringent conditions (*16*). Lanes 1 and 3, RNA from PMA-stimulated cells; lane 2, RNA from control cells. (**A**) Hybridization with human SAA probe and (**B**) total cellular RNA stained with ethidium bromide.

Alternatively, they may amplify the signal for collagenolysis from low levels of Il-1, TNF, or substance P. We suggest that these proteins may play a role in modulating connective tissue breakdown in both normal and disease states.

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9. Of the four proteins isolated, the species *p*I 8, 12 kD is the least abundant. The 14-kD SAA-like protein often predominates at *p*I 8, whereas the *p*I 5 material often contains a more equal mixture [see (*4*) for details].
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14. This increased hybridization is not an artifact of gel-loading for three reasons. (i) Figure 5B shows that approximately equal amounts (25 μg) of whole-cell RNA were loaded in each lane; (ii) when this same blot was probed with a cDNA clone for stromelysin-proactivator (*3*, *7*), a metalloproteinase whose transcription is increased by PMA (*3*), large increases in stromelysin mRNA were seen in lanes 1 and 3, which contain RNA from PMA-stimulated cells (*3*); and (iii) Fig. 1A shows increased proteins corresponding in molecular size to SAA-like proteins in PMA-stimulated cells compared to control cells.
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A cAMP-Regulated Chloride Channel in Lymphocytes That Is Affected in Cystic Fibrosis

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A defect in regulation of a chloride channel appears to be the molecular basis for cystic fibrosis (CF), a common lethal genetic disease. It is shown here that a chloride channel with kinetic and regulatory properties similar to those described for secretory epithelial cells is present in both T and B lymphocyte cell lines. The regulation of the channels by adenosine 3′,5′-monophosphate (cAMP)–dependent protein kinase in transformed B cells from CF patients is defective. Thus, lymphocytes may be an accessible source of CF tissue for study of this defect, for cloning of the chloride channel complex, and for diagnosis of the disease.

AN UNDERLYING MOLECULAR BASIS for CF involves an unusual Cl^- selective channel with distinctive properties in the apical membrane of the epithelial cells (*1–6*). Activation of the Cl^- channel by cAMP-dependent phosphorylation is defective in CF, implying an altered phosphorylation of the ion channel or of a regulatory protein or an altered response to the phosphorylation. Our observations in lymphocyte cell lines initiated a specific study of whether the CF-affected Cl^- channel also resides in these cells.

We first used Jurkat E6-1 human T lymphocytes (*7*). Single-channel Cl^- currents were recorded by the extracellular patch-clamp technique (*8*) from inside-out and cell-attached patches. Activity of the Cl^- channel was first identified by excising

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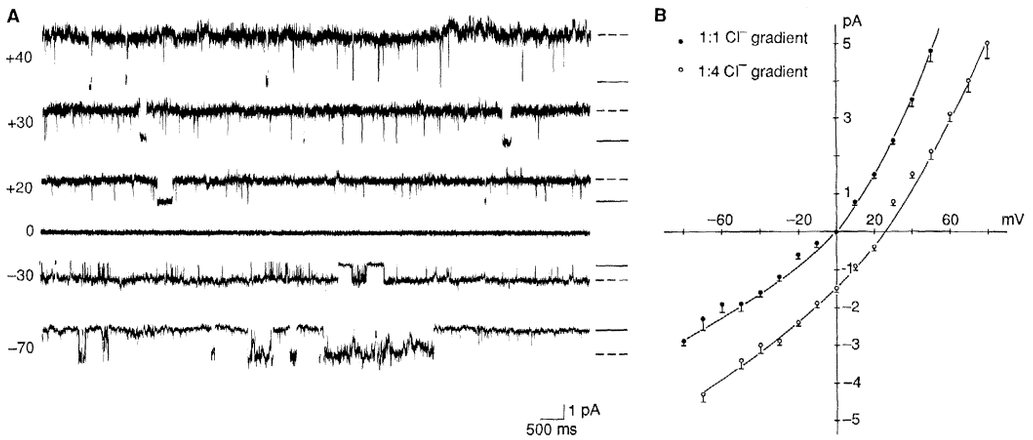

Fig. 1. Tracings and *I-V* relations of Cl^- channels in Jurkat E6-1 T lymphocytes. (**A**) Single Cl^- channel currents recorded from an excised, inside-out patch at six holding voltages. The polarity of transmembrane voltages is expressed with reference to the pipette solution. Outward currents are upward deflections. Baseline current level is indicated by the solid line and open-channel current level by the dashed line at the right of each trace. Bath and pipette solutions were identical, consisting of 154 m*M* NaCl, 5.5 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, and 10 m*M* Hepes-NaOH (*p*H 7.3). Records were filtered at 1 kHz. (**B**) Current-voltage relation obtained from the channel shown in (A) (●) and a channel recorded from a patch in the same bath solution, but with a pipette containing 37.5 m*M* NaCl and 5 m*M* Hepes-NaOH (*p*H 7.4) (○). Imposition of the approximate 1:4 Cl^- gradient shifted the reversal potential by +28 mV (Nernst potential predicted shift is +36 mV for a perfectly Cl^- selective channel). Cells were washed twice, resuspended in the bathing solution, and maintained at room temperature (20° to 22°C) for recording with a List-EPC7 patch-clamp amplifier. Current signals were stored on videocassette tape and later digitized for analysis. Current amplitudes for the *I-V* relation were estimated by the user intervention mode of the single-channel analysis program IPROC-2 and NFITS (C. Lingle and F. Sachs). Error bars represent SDs based on $n = 50$ amplitudes measured per potential.

membrane patches into Hepes-buffered Ringer's solution and imposing large depolarizing potentials (Fig. 1A). Under these conditions, Cl^- channel activity appeared in 11/14 (79%) of patches after a time varying between 10 s and 10 min. The Cl^- channel currents were characterized by nonlinear current-voltage (*I-V*) relations in symmetrical solutions, with slope conductance higher at depolarized voltages (Fig. 1B). Slope conductance at 0 mV was approximately 40 pS (39 ± 7.3 pS, mean ± SD, $n = 8$). Although conductance was somewhat variable from patch to patch, the kinetic appearance of prolonged bursts of well-resolved openings at 1-kHz recording bandwidth, interrupted by brief flickers as well as intermittent long closures, and the strong outward rectification of the *I-V* relation distinguished the Cl^- channel from other T lymphocyte channels. By imposing an approximate 1:4 Na^+ and Cl^- gradient and documenting +28-mV shift of reversal potential (Fig. 1B), we determined that the channel was a predominantly anion-selective channel. Calculation of the relative Cl^- to Na^+ permeability, *P* (Cl/Na), from the Goldman-Hodgkin-Katz equation yields a value of approximately 10:1, which is identical (*4*) or similar (*3*) to published values for the epithelial cell Cl^- channel.

The relation of channel activation in excised patches to voltage and $[Ca^{2+}]_i$ was complex. The variable time to appearance of Cl^- currents after depolarization (seconds to minutes), the persistence of channel opening at hyperpolarized potentials once activated by depolarization, and the occasional (1/11 or 8%) activation of patches subjected to sustained hyperpolarizing potentials indicate that the mechanism of Cl^- channel gating in excised patches is distinct from many voltage-gated Na^+, K^+, and Ca^{2+} channels. The relation of lymphocyte Cl^- channels to voltage appears similar to that described for epithelial cell Cl^- channels (*3*, *4*). Lymphocyte Cl^- channels required a minimum of approximately $10^{-7}M$ $[Ca^{2+}]_i$, in which 4/17 (24%) of tested patches were

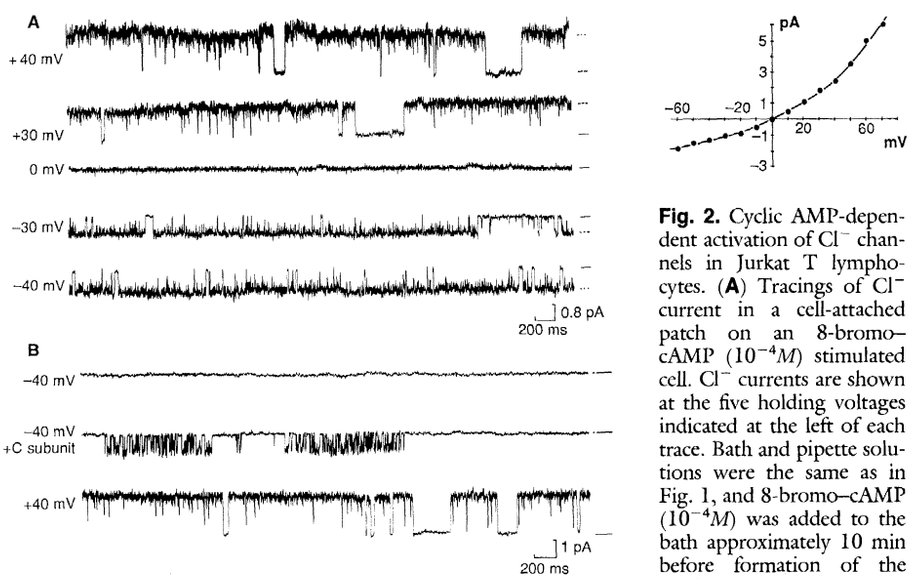

Fig. 2. Cyclic AMP-dependent activation of Cl^- channels in Jurkat T lymphocytes. (**A**) Tracings of Cl^- current in a cell-attached patch on an 8-bromo–cAMP ($10^{-4}M$) stimulated cell. Cl^- currents are shown at the five holding voltages indicated at the left of each trace. Bath and pipette solutions were the same as in Fig. 1, and 8-bromo–cAMP ($10^{-4}M$) was added to the bath approximately 10 min before formation of the patch. (Inset) Current-voltage relation for this channel. Voltages are expressed relative to cellular resting potential. The calculated slope conductance at reversal potential was 45 pS. (**B**) Effect of catalytic subunit of cAMP-dependent protein kinase on a Cl^- channel. Tracings are from an excised, inside-out patch. Baseline current level is indicated by a solid line at the right of each, and holding potential is indicated at the left of each trace. During the first 3 min after excision of the patch into a bath containing 1 m*M* ATP, no channels opened (trace 1). Subsequent addition of 150 n*M* of purified catalytic subunit activated the channel at the −40 mV (trace 2). Channel activity continued when the patch was depolarized to +40 mV (trace 3). The bath solution consisted of 140 m*M* NaCl, 2 m*M* $MgCl_2$, 0.1 m*M* $CaCl_2$, 1.1 m*M* EGTA (calculated free Ca^{2+} $10^{-8}M$), and 10 m*M* Hepes-NaOH (*p*H 7.2). The pipette solution consisted of 154 m*M* NaCl, 5.5 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* MgCl, 10 m*M* Hepes-NaOH (*p*H 7.3). The catalytic subunit of the cAMP-dependent protein kinase was purified to homogeneity from bovine heart essentially as described (*13*). The cAMP kinase stock (10 μ*M*) is in 25 m*M* KPi (*p*H 6.7), containing 10 m*M* dithiothreitol and 50% glycerol (w/v). The specific activity is 1.2 μmol/min per milligram with histone as substrate. Addition of an equal volume of vehicle without the catalytic subunit had no effect on eight tested patches. Addition of 1 m*M* ATP alone induced channel activation in 2/20 patches, but only after sustained depolarization by +80 mV. The latter finding was attributed either to endogenous kinase or to the effects of sustained depolarization.

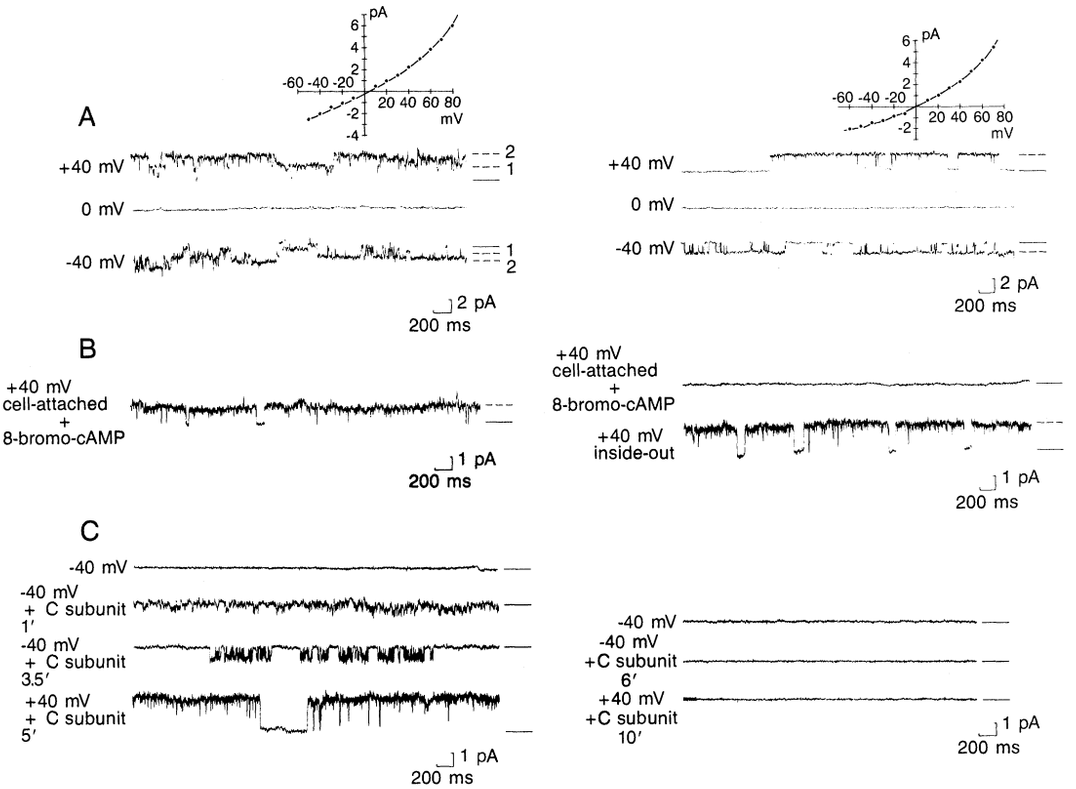

Fig. 3. Cl^- currents in normal and CF-derived mutant human lymphoblasts. (**A**) Tracings and current-voltage relations of single channel Cl^- currents recorded from excised inside-out patches from normal (left trace) and CF-derived (right trace) human lymphoblasts. Recording conditions were as in the legend to Fig. 1A. At least two channels are active in the normal lymphoblast patch. (**B**) Effect of 8-bromo–cAMP ($10^{-4}M$) stimulation. (Left) Outward Cl^- currents at +40 mV recorded in a cell-attached patch of a normal lymphoblast after exposure to 8-bromo–cAMP. (Right) Upper trace shows lack of Cl^- current activity in a cell-attached patch of a CF-derived human lymphoblast exposed to 8-bromo–cAMP. After excision of the patch and depolarization by +40 mV, typical Cl^- current activity is then recorded (right lower trace). Bath and pipette solutions are the same as for Fig. 1A. Voltages are expressed relative to cellular resting potential in the cases of the cell-attached patches. (**C**) Effect of catalytic subunit of cAMP-dependent protein kinase on Cl^- channels. Tracings are from excised, inside-out patches from normal (left) and CF-derived (right) lymphoblasts. Bath and pipette solutions are the same as for Fig. 2B. In the case of the normal (left), no channels opened during the first 3 min after excision of the patch into a bath containing 1 m*M* ATP (trace 1). Subsequent addition of 150 n*M* of purified catalytic subunit activated the channel within 1 min at the hyperpolarized voltage (trace 2). Channel activity after 3.5 min at −40 mV (trace 3) and at +40 mV (trace 4). In the CF-derived lymphoblast patch (right), no channel activity was seen under any of these conditions.

activated, compared to 0/11 at $10^{-8}M$ $[Ca^{2+}]_i$ and 0/10 at approximately $10^{-9}M$ $[Ca^{2+}]_i$ (*9*). Once activated, however, there was no apparent effect on channel gating when the bath solution was lowered to $10^{-9}M$ $[Ca^{2+}]_i$ (*10*). This seems to contraindicate a direct ligand-gating mechanism by Ca^{2+}, such as typified by the Ca^{2+}-activated K^+ channel (*11*). The apparent dependence of lymphocyte Cl^- channel activation in excised patches on a minimum of $10^{-7}M$ $[Ca^{2+}]_i$ distinguished them from epithelial cell Cl^- channels, which can be activated by depolarization in excised patches in $10^{-8}M$ $[Ca^{2+}]_i$ (*5, 6*).

Other than the noted exception, the properties of the Cl^- channel in Jurkat T lymphocytes were similar to those of the Cl^- channel in the apical membrane of airway epithelial cells (*3, 4*). We determined whether the T lymphocyte Cl^- channel is also regulated by a cAMP-dependent process. We compared recordings from cell-attached patches of control and 8-bromo–cAMP–treated cells. In control conditions, Cl^- currents were never seen at recording potentials equal to or more hyperpolarized than resting potential. The Cl^- channel could infrequently (3/13 cell-attached patches or 23%) be induced under conditions of imposed depolarization (+80 mV) sustained for periods up to 20 min. In contrast, we recorded Cl^- current activity from 14/20 cell-attached patches (70%) of cells preexposed to 8-bromo–cAMP ($10^{-4}M$) for between 5 and 30 min (Fig. 2). Sustained depolarization was not a necessary requirement for activation by 8-bromo–cAMP–dependent mechanisms. The *I-V* relation, slope conductance at reversal potential, and kinetic appearance of Cl^- currents evoked by 8-bromo–cAMP were similar to Cl^- currents induced by depolarization in excised patches (Fig. 2A).

We then exposed the cytoplasmic surface of excised patches to a catalytic subunit of cAMP-dependent protein kinase plus 1 m*M* adenosine triphosphate (ATP). Patches were held at either −40 mV or +40 mV in $10^{-8}M$ $[Ca^{2+}]_i$, conditions in which no detectable channel openings were seen in a control series of recordings. Addition of the catalytic subunit (50 to 150 n*M*) plus 1 m*M* ATP evoked Cl^- currents within seconds to 4 min in 11/23 tested patches (Fig. 2B). These results are consistent with activation of Cl^- channels by a phosphorylation event by cAMP-dependent protein kinase.

We determined whether the CF defect in cAMP-dependent phosphorylation of chloride channels (*3–6*) was present in the lymphocyte channel. We compared normal human Epstein Barr virus (EBV)–transformed (B cell) lymphoblasts to mutant human EBV-transformed lymphoblasts derived from CF patients (*12*). First we confirmed that a Cl^- channel of similar properties was present in the lymphoblasts. Single channel Cl^- currents were recorded from inside-out patches excised into Hepes-buffered Ringer's solution and depolarized for substantial periods (seconds to minutes) by +40 to +80 mV. Channels were recorded from 5/9 (56%) normal lymphoblasts and 13/23 (57%) CF lymphoblasts (Fig. 3A). The selectivity of the channel for Cl^- over cations was confirmed by the predicted positive reversal shift in a 1:4 Cl^- gradient ($n = 1$ for normal; $n = 7$ for CF). The persistence of channel opening at all potentials after depolarization-induction and the kinetic appearance of prolonged bursts of openings were indistinguishable from that described for the Jurkat T cell. No difference in Cl^- channel activity induced by depolarization in excised patches could be discerned between normal and CF-derived lymphoblasts.

We then sought to determine whether the B lymphoblast channel is also regulated via a cAMP-dependent process and whether this is defective in CF. We recorded typical Cl^-

current activity from 7/14 (50%) cell-attached patches of normal B lymphoblasts exposed to 8-bromo–cAMP ($10^{-4}M$) (Fig. 3B). In contrast, Cl^- current activity was never seen in 13 (0%) of cell-attached patches of CF lymphoblasts after exposure of the cell to 8-bromo–cAMP ($10^{-4}M$). In two cases, including the example depicted in Fig. 3B, we were able to excise the patch after a 10-min recording period in the cell-attached configuration. In both cases, typical current activity was induced after patch excision and depolarization. Thus, at least in these two cases, Cl^- channels were present in the patches of CF cells but were unable to be activated by the cAMP-dependent pathway.

In a final protocol we compared the effect of 150 n*M* catalytic subunit of cAMP-dependent protein kinase plus 1 m*M* ATP on excised patches of normal versus CF-derived B lymphoblasts. The protocol was identical to that described for Jurkat T lymphocytes. Under these conditions Cl^- current activity was induced in 5/11 (45%) of normal B lymphoblast inside-out patches after exposure to catalytic subunit plus ATP (Fig. 3C). Activation occurred within a few seconds to 2 min after addition of catalytic subunit to the bath. In contrast, Cl^- current activity was never recorded in 22 (0%) inside-out patches of CF cells exposed to catalytic subunit plus ATP in an identical protocol (Fig. 3C).

In summary, we have presented evidence that a cAMP-regulated chloride channel similar to that described for airway epithelial cells (*3–6*) is present in both T and B lymphocyte cell lines. Importantly, the genetically programmed defect in the regulation by cAMP-dependent phosphorylation described for CF appears to be present in transformed lymphocytes as well as epithelial cells. The finding of CF-affected Cl^- channels in lymphocytes, which are easily accessible and abundant, may have important implications for the pathophysiology and for the future biochemical and molecular genetic analysis of the disease.

Note added in proof: While extending our studies we have noticed large variations in the percentage of lymphocyte patches with detectable Cl^- channel currents that may be due to different lots of fetal calf serum.

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Transformation and Plasmacytoid Differentiation of EBV-Infected Human B Lymphoblasts by *ras* Oncogenes

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The biological effects of *ras* oncogene activation in B cells were studied by using amphotropic retroviral vectors to introduce H- or N-*ras* oncogenes into human B lymphoblasts immortalized by Epstein-Barr virus. Expression of both H- and N-*ras* oncogenes led to malignant transformation of these cells, as shown by clonogenicity in semisolid media and tumorigenicity in immunodeficient mice. In addition, terminal differentiation into plasma cells was detectable as specific changes in morphology, immunoglobulin secretion, and cell surface antigen expression. This combined effect, promoting growth and differentiation in human lymphoblasts, represents a novel biological action of *ras* oncogenes and has implications for the pathogenesis of terminally differentiated B-lymphoid malignancies such as multiple myeloma.

ONCOGENE FUNCTION HAS BEEN mainly assessed by in vivo and in vitro studies whose endpoint is the production of a transformed phenotype in fibroblasts (*1*). Although of great value for our approaches to oncogenesis, these studies may not adequately reflect the complex alterations of both growth and differentiation that characterize the phenotype of naturally occurring malignancies in other tissue types. It is conceivable, and in part already proved, that distinct biological effects of different oncogenes and phenotypic changes specific for certain lineages or stages of differentiation may become apparent only by studying different tissue types.

The lymphoid tissues represent a particularly attractive model system for studying the biological effects of different oncogenes, since pathways controlling growth, lineage commitment, and differentiation of lymphoid cells can be dissected by use of a vast array of available molecular and immunological reagents. We established an in vitro transformation assay in which Epstein-Barr virus (EBV)–immortalized B lymphoblastoid cells (LCL) are used as targets for testing the biological activity of oncogenes (*2*). Using this system, we showed that the expression of c-*myc* oncogenes induces malignant transformation of these cells without relevant changes in their differentiation phenotype (*2*). We now describe the biological effects of activated H-*ras* and N-*ras* oncogenes (*3*) in the same target cells. These oncogenes also induced malignant transformation in LCL but in addition lead to dramatic phenotype changes involving terminal differentiation of lymphoblasts into plasmacytoid cells.

An activated H-*ras* allele derived from Harvey sarcoma virus (*4*), or a human N-*ras* allele, activated by a mutation at codon 12 (*5*), were introduced into LCL derived from normal cord blood (CB33 line) or from

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