egg-bearing screens. Of course, the function of the calcium transport is very different. The chorioallantoic epithelium is involved in moving calcium from the shell to the embryo. Thus, its function lies in producing changes external to the epithelial cells themselves.

What is the role of the calcium current in the polarizing *Pelvetia* egg? We believe that it is the creation of an intracellular gradient of free calcium ions, with the higher concentration being at the leaking end. We are encouraged in this view by the finding (*11*) that there is an unequal distribution of calcium along the axis of the slug of the cellular slime mold *Dictyostelium discoideum*, with more calcium found in the anterior prestalk region. It was also shown that an increased level of extracellular calcium induced stalk formation and inhibited spore formation, which suggests that calcium does control the developmental fate of these cells.

One way in which a calcium gradient might act to polarize these eggs is by producing an electric field across the cytoplasm. The magnitude of the gradient—and hence the field—depends on the concentration of fixed calcium-binding sites and mobile calcium-binding molecules. We have shown elsewhere (*9*), using estimates from other systems for these quantities, that a calcium current of 0.03 $\mu a/cm^2$ could produce a cytoplasmic field across a *Pelvetia* egg of 0.1 volt/cm or more. Such fields are quite large enough to segregate cytoplasmic components (*3*).

One can envision other ways in which a cell might make use of a calcium gradient to bring about polarization. The control of contractility by calcium ion is well established, and extends even to primitive systems; Taylor *et al.* (*12*) have shown that cytoplasmic contractility of amoebas may be regulated by the level of free calcium ion. Another effect of calcium has been demonstrated by Weisenberg (*13*). Working with rat brain tubulin, he found that a free calcium concentration of $6 \times 10^{-6} M$ blocked the repolymerization of microtubules, and he concluded that "calcium appears to be a logical candidate as a regulator of microtubule polymerization *in vivo*."

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Spontaneous Regression of Friend Virus Induced Leukemia: Coinfection with Regressing and Conventional Strains of Virus

Abstract. *Mixtures of Friend virus (CFV) and the regressing strain of Friend virus (RFV) induce leukemia which regresses. The dominance of the regressing phenotype is solely a function of a threshold dose of RFV. The minimum amount of RFV which induced regression of CFV leukemia is below the titer for induction of Friend disease, but does correlate with the titer of lymphocytic leukemia (helper) activity in these stocks.*

The systematic study of spontaneous cancer regression, some 200 cases of which have been documented for the human disease, has been stimulated by the recent availability of suitable animal models. Friend virus leukemia is a progressive neoplastic disease characterized by massive splenomegaly leading inevitably to splenic rupture and death (*1*).

We have reported the isolation and characterization of a virus strain capable of inducing a disease initially indistinguishable from that induced by Friend or Rauscher leukemia virus (*2*). The disease induced by this agent differs from that initiated by conventional leukemia viruses in that it appears to be self-limiting. The characteristic splenic response, histologically indistinguishable from conventional Friend virus leukemia, does not lead to death; rather, in a significant proportion of leukemic mice, the massive proliferation of splenic cells is reversed, and the organ returns to normal architecture and mass. The virus synthesis which accompanies the disease similarly subsides.

In studies carried out shortly after the initial isolation of RFV, coinfection with both the conventional Friend virus (CFV) and the regressing Friend virus strain (RFV) did not demonstrate a marked influence of RFV on CFV induced leukemia (*3*). Subsequent sug-

Table 1. Spontaneous regression of leukemia induced by regressing and conventional strains of Friend leukemia virus.

Virus inoculation			Incidence		
CFV	RFV	Ratio	Leukemia (No. leukemic/ No. inoculated)	Regression (No. regressed/ No. leukemic)	Percent
100*	—		11/11	0/11	0
10	—		9/10	1/ 9	11
1	—		5/10	0/ 5	0
0.1	—		1/10	0/ 1	0
—	776		10/10	2/10	20
—	78		10/10	2/10	20
—	7.8		7/10	7/ 7	100
—	0.8		6/10	6/ 6	100
25	388	0.06	9/10	2/ 9	22
25	39	0.64	10/10	9/10	90
25	3.9	6.41	10/10	6/10	60
25	0.4	64.1	9/10	4/ 9	44
2.5	388	.006	10/10	3/10	30
2.5	39	.06	9/10	4/ 9	44
2.5	3.9	.64	9/10	6/ 9	67
2.5	0.4	6.4	7/ 9	1/ 7	14

* Leukemic dose, 50 percent: (that dose cf virus which will induce leukemia in 50 percent of inoculated weanling Swiss/ICR mice within 21 days).

gestion of an immunological mechanism for leukemia regression (*4*) indicated the need for a more thorough evaluation of potential interactions.

Virus stocks were prepared as 20 percent (weight to volume) cell-free homogenates of leukemic spleens in phosphate buffered saline and stored at −70°C. Random bred ICR/Ha Swiss weanling male mice were inoculated intraperitoneally with a total of 0.5 or 1.0 ml of virus or mixture of viruses, and palpated twice weekly for splenomegaly. Palpations were monitored at intervals by determining spleen weights of animals that were killed for that purpose; spleen weights have been shown to correlate precisely with pathological changes and virus titers in the spleen and peripheral blood.

Groups of ten mice were inoculated with 42 different combinations of RFV and CFV. The total virus dose of RFV plus CFV ranged from 0.1 to 776 median leukemic doses (LD_{50}'s). The ratio of CFV to RFV in mixtures varied from 6.2×10^{3} to 6.0×10^{-3}. A portion of this data may be seen in Table 1. If all of the combinations are divided into three categories, namely, those infected with RFV alone, CFV alone, and combinations of the two, then the percentage incidences of regression for the three are 56, 2, and 20, respectively. Mice infected with RFV alone showed the expected incidence of leukemia regression (*2*). The 2 percent incidence of regression in mice infected with CFV alone was also within normal limits. Of primary interest was the observation that coinfection with CFV and RFV resulted in a very significant incidence of spontaneous leukemia regression. It should be noted that each of the combinations of CFV and RFV contained at least 2.5 LD_{50}'s of nonregressing CFV.

The combinations of CFV and RFV comprised varying absolute and relative doses of the two viruses. It was therefore possible by correlation analysis (linear regression) to determine if the incidence of regression were a function of the absolute dose of RFV, of CFV, or of the ratio of the two independent of the absolute dose of each. None of these were found to be operative. Instead, regression appeared to depend only on a threshold dose of RFV.

To characterize this phenomenon, a large series of experiments were carried out in which inoculation of CFV, which only very rarely regresses, was admixed with various amounts of RFV (Table 2). The leukemia regression in these mice was identical to that observed in mice receiving RFV alone. As was previously observed (*2*) leukemia regression does not occur in all animals inoculated with RFV. The 22 to 47 percent incidence of regression (Table 2) may be compared with a 30 to 75 percent incidence of leukemia regression observed with dozens of RFV preparations over a period of several years.

Table 2. Influence of regressing leukemia virus on the spontaneous regression of leukemia induced by conventional Friend leukemia virus.

Inoculum and dilution	No. leukemic/ No. inoculated	Incidence of regression	
		No. *	Percent†
CFV^{-2}	18/20	0	0
RFV^{-1}	19/20	9	47
CFV 10^{-2} + RFV 10^{-1}	10/10	3	30
CFV 10^{-2} + RFV 10^{-3}	10/10	3	30
CFV 10^{-2} + RFV 10^{-5}	9/10	3	33
CFV 10^{-2} + RFV 10^{-7}	9/10	2	22
CFV 10^{-2} + RFV 10^{-9}	9/10	0	0
CFV 10^{-2} + RFV 10^{-11}	8/ 9	0	0

* Number of animals in which leukemia developed and subsequently regressed. † Percent regression = (number of animals regressed/number leukemic) × 100.

The titer of the efficacy of RFV in causing the regression of CFV induced leukemia is substantially higher than the leukemogenic activity of these stocks of virus. As shown in Table 2, regression was observed when RFV, diluted to as much as 10^{-7}, was inoculated admixed with a leukemogenic dose of CFV. Leukemogenic activity for these and other preparations of either RFV or CFV is rarely observed beyond a dilution of 10^{-4}. This influence of RFV in mixtures of CFV and RFV at dilutions a number of orders of magnitude greater than the minimum infectivity dose has been a consistent feature of all of the experiments so far.

The Friend leukemia virus complex consists of two components: a defective spleen focus forming virus (SFFV) and a lymphocytic leukemia virus (LLV). The LLV possesses helper activity for the formation of foci by SFFV in spleens of inoculated mice. The development of typical Friend disease is a function of the SFFV component (*5*). Helper activity in Friend virus preparations has been reported to be effective at dilutions 100-fold or more greater than the maximum titer for the induction of Friend leukemia (*6*). The ability of subleukemogenic amounts of RFV to induce regression of the disease initiated by CFV calls attention to this similarity between RFV preparations and LLV in this respect and suggests that the difference between RFV and CFV may be a function of their LLV's, the LLV of RFV being responsible for the property of regression.

While the mechanism of regression is not yet well understood, it has been established that the immune system of the host must be intact in order for regression to occur (*4*). The dominance of the regressing phenotype in mixtures of CFV and RFV, coupled with the suggested immunological basis for regression, suggests that the difference between the two viruses may reside in the immunogenicity of the cells infected by them. Leukemic cells induced by inoculation with RFV may be much more efficient in inducing an effective immune response than CFV. Our data suggest that the diseases induced by CFV and RFV are both sensitive to the host response initiated by RFV. The specific characterization of the nature of this antineoplastic response and its range remain to be studied.

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