toms of virus infection appear. The resistance of red clover to powdery mildew following infection by BYMV seems to represent a type of interaction distinct from those previously reported. In the present case the fungus spores germinated and induced a hypersensitive reaction in the virus-infected host but not in the virus-free host. Apparently, virus infection of this mildew-susceptible clover alters the metabolism of the host in such a way that it responds to mildew infection in the same way as resistant types.

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Antigens Associated with a Tumor Virus: Rejection of Isogenic Skin Grafts from Leukemic Mice

Abstract. *Skin grafts from leukemic adult Balb/c mice inoculated when newborn with a leukemia virus, showed evidence of rejection when transplanted to normal Balb/c mice or genetically compatible F_1 hybrids. Therefore, the "cellular" antigens associated with oncogenic viruses may not be limited to the induced tumors.*

Tumors induced by oncogenic viruses possess newly acquired antigen or antigens capable of eliciting transplantation immunity in isogenic hosts. Studies by numerous investigators have shown that the antigens are virus associated and are not present in the normal host complement. These antigens have been well documented in the case of the Gross, Friend, Moloney, and Rauscher leukemia viruses (*1*). In general, all tumors induced by a given virus seem to share at least one antigen in common; therefore, specificity is determined by the virus rather than the individual tumor. Since the occurrence of a "new cell antigen" suggests several theoretical implications including an immunologic approach to the therapy of these tumors, it is of primary importance to determine if the antigens are confined to the induced tumors. We now present evidence that the presumably normal skin of leukemic mice also contains a virus-associated antigen.

Newborn Balb/cDe mice were injected subcutaneously with concentrates obtained from high-speed centrifugation of a leukemia virus originally isolated from C3H plasma cell tumor 70429. Previous studies have shown that transplants of tumors induced by this virus are antigenic in isogenic mice (*2*). The mice were examined weekly for evidence of leukemia—that is, enlarged spleen, inguinal lymph nodes, and thymus glands. Mice that displayed these symptoms were used as skin graft donors. In addition, grafts from normal, noninjected, Balb/c donors were made as a control for genetic homogeneity. About one-third of the host mice received grafts from both normal and leukemic donors; the remainder received single grafts. Grafts were made by a method similar to one previously described (*3*) in all combinations except male to female, and the direction of hair growth was altered with respect to that of the host. Recipient mice were either Balb/c or (Balb/c × DBA/2e)F_1 hybrids, and approximately half were immunized prior to grafting by one or more subcutaneous injections of about 7×10^5 cells from a tumor induced by this virus in a Balb/c mouse. The time between the last immunization and grafting averaged 21 days. The few mice that developed progressively growing tumors from this dosage of cells were discarded. Grafts were observed three times a week for the first month, and then once a week for a minimum of 100 days. Those that appeared to be undergoing rejection were observed more frequently. Skin graft survival was evaluated by gross appearance,

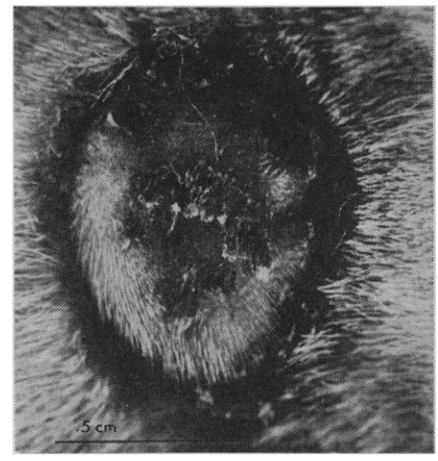

Fig. 1. Skin graft from Balb/c donor with virus induced leukemia on nonimmune Balb/c host after 14 days, showing areas of progressive necrosis.

particularly that of the epidermis, and all grafts were placed in one of three categories as follows: (i) complete survival—little or no evidence of rejection; (ii) partial rejection—destruction of more than 50 percent of the graft area; (iii) complete rejection—apparent destruction of all viable graft elements.

All skin grafts from normal Balb/c donors both on normal and on immune Balb/c and (Balb/c × DBA/2)F_1 hybrid hosts healed into place uneventfully and showed no signs of rejection throughout the duration of the experiments (Table 1). Fourteen grafts from leukemic donors were, however, apparently completely rejected by their hosts. The average survival time of those grafts rejected by immune hosts was 18 days. Of all grafts on nonimmune hosts only one was completely rejected, and this survived 23 days. The pattern of rejection was similar to that observed with skin homografts involving minor degrees of genetic in-

Table 1. Rejection of skin grafts from normal and leukemic Balb/c mice on genetically compatible Balb/c and (Balb/c × DBA/2)F_1 hybrids.

Host	Status of host*	Total grafts	Rejections		Tumors at graft site
			Complete	Partial	
		Leukemic Balb/c graft donors			
F_1 hybrid	I†	25	6	4	0
F_1 hybrid	NI	31	1	5	14
Balb/c	I	24	7	8	0
Balb/c	NI	19	0	6	8
		Normal Balb/c graft donors			
F_1 hybrid	I	23	0	0	0
F_1 hybrid	NI	22	0	0	0
Balb/c	I	27	0	0	0
Balb/c	NI	10	0	0	0

* Immune mice previously received one or more subthreshold doses of cells from a tumor induced by the virus. † I, immune; NI, nonimmune.

compatibility, and the immunologic reactivity of both hybrid and inbred hosts to the grafts was about equal. One or more necrotic areas appeared on graft surfaces about 2 weeks after grafting (Fig. 1); these would enlarge and coalesce to include the entire graft surface. When rejection was complete, all that remained was a dermal collagen pad, somewhat reduced in size, overlaid with host epithelium in which hair growth usually remained absent. The partial rejection of grafts began in the same fashion except that, after varying amounts of graft destruction, which reached a maximum at 19.8 and 21.3 days in immune and nonimmune mice respectively, progressive necrosis would cease, and these areas would heal. Once healing had occurred graft size was usually reduced, and no further evidence of incompatibility was observed; this left the impression that only certain groups of cells were antigenic. The occurrence of partial rejection was about equally distributed between immune and nonimmune hosts. Small amounts of necrosis were seen in many grafts taken from leukemic donors, but these grafts have not been included in the table because of their doubtful significance.

Tumors often developed under grafts on nonimmune hosts after an average time of 46 days. Eight of these tumors that arose in hybrid hosts were subjected to transplantation studies which showed them to be of donor origin. This observation, in addition to the fact that this virus does not induce solid tumors at the site of inoculation, indicates that these tumors arose from leukemic cells present in donor skin at the time of grafting, rather than being virus induced subsequently. The response of immune hosts was probably sufficient to destroy these cells before tumors could develop.

The immunologic nature of this skin graft rejection is evidenced by the significantly larger ($P < .001$) number of complete graft rejections in immune as opposed to nonimmune hosts. It is possible that graft rejection is a consequence of the destruction of leukemic cells within the graft. There is some evidence against this possibility in that skin grafts from weanlings, injected as newborns with virus and showing no overt signs of leukemia, are similarly rejected in the apparent absence of leukemic cells. Thus there appears to be an antigen common to both the skin of leukemic mice and the induced tumor that elicits immunity in normal Balb/c mice. Thus, in animals infected with a leukemia virus, the cells of other "normal" tissues may also acquire virus-associated antigenic properties. This is supported by the reported occurrence of virus particles in the mammary glands of nonleukemic C3H(f) females injected with passage A leukemia virus (*4*). We used skin grafts because of the ease with which their survival can be followed, but whether grafts of other tissues might also show evidence of rejection is undetermined.

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Reversible Sonic Inhibition of Protein, Purine, and Pyrimidine Biosynthesis in the Living Cell

Abstract. *Yeast cells, carrying on normal biosyntheses in the treatment cup of a sonic oscillator, cease synthesis when the oscillator is operated at a critical low power level. Synthesis is resumed immediately when the oscillator is turned off. At the sonic intensities employed the cells are not extracted, uptake of ammonium ion is unaffected, and most cells remain viable. The inhibition may be the result of disruption of supramacromolecular organization in the cell.*

The disruption of cells by sound has been widely used as a technique for extracting enzymes and other components. Different structures of the cell may be broken down at different rates; certain enzymes may be extracted from the cell at different rates in sonic fields (*1*) and in the Mickle disintegrator (*2*). The attachment of respiratory enzymes in *Azotobacter* to membrane envelopes within the cell has been demonstrated in this way (*2*). Hughes and Nyborg (*3*) have demonstrated that cells can be disrupted under conditions whereby treatment with sound ("sonication") produces gas microbubbles, but the collapse of the bubbles, which produces cavitation, is suppressed. In experiments in which a vibrating needle was placed next to cell walls, violent microeddies could be seen in large cells with intact cell walls. This phenomenon provides a mechanism whereby one might be able to alter the spatial arrangement of organelles and fragile compartments without necessarily destroying the cell. If the several steps of a given biosynthetic pathway proceed efficiently in the cell only when the required enzymes are arranged or compartmentalized in a certain order, disruption of that order would be expected to inhibit the biosynthesis. On the basis of these ideas an investigation was undertaken to determine if the biosynthesis of certain compounds in the intact cell could be inhibited by sound at a frequency of 10 kc/sec.

The yeast employed was a haploid strain of *Saccharomyces cerevisae*, S1237, with biochemical deficiencies for adenine, uracil, and histidine. Aminoimidazole ribotide (AIR) accumulates in this strain in the absence of adenine as the result of a genetic block of purine synthesis (*4*). The biosynthesis of purines proceeds normally up to the reaction in which AIR is produced. When the yeast is grown in the presence of adenine, synthesis of AIR is inhibited, but when adenine is removed AIR synthesis proceeds at a high rate. The assay for AIR has been described (*4*). The block in the pyrimidine pathway leads to the accumulation of ureidosuccinic acid and dihydroorotic acid in the absence of uracil (*5*). These compounds were assayed by the Gerhart and Pardee modification (*6*) of the Koritz and Cohn carbamylamino tests; dihydroorotic acid is converted to ureidosuccinic acid in the assay. Protein was determined by use of the phenol reagent (*7*). The Raytheon 250-w 10 kc sonic disintegrator was used for sonic treatment. From a thermostatically controlled refrigerated bath, water at 30°C was circulated through the jacket of the sonic cup. The temperature of the cell suspension in the sonic cup was measured with a thermistor device during and after soni-