Resistance to Erysiphe polygoni of Red Clover Infected with Bean Yellow Mosaic Virus

Abstract. *The development of* Erysiphe polygoni *on leaves of red clover was retarded by poor infection with bean yellow mosaic virus. Conidia germinated on leaves infected with the virus but disintegrated following necrosis of the leaf tissue in the immediate area of the germinating conidia.*

Increased resistance or susceptibility of plant tissues to a pathogen resulting from prior infection by another pathogen has been reported by several investigators. In bean plants infected with the rust fungus, *Uromyces phaseoli typica*, more tobacco mosaic virus (TMV) was recovered from rusted than from non-rusted leaves, whereas the rust fungus sporulated less vigorously on infected leaves than on virus-free leaves (*1*). A later report indicates that rust infection in bean reduces the susceptibility of the tissue to infection by TMV as judged by number of local lesions produced; and that TMV infection reduces susceptibility to the rust fungus, the reduced susceptibility resulting from inhibition of spore germination (*2*). A somewhat similar interaction was reported between *Erysiphe polyphaga* and cucumber mosaic virus in the cucumber plant. Symptoms of the virus in plants infected with the mildew fungus were relatively mild, while the healthy portions of virus-infected leaves were more susceptible to mildew than the portions bearing lesions (*3*).

It was observed in this laboratory that the development of *Erysiphe polygoni*, a species of powdery mildew, is almost totally retarded on the leaves of Kenland red clover (Ky C 36) systemically infected with bean yellow mosaic virus (BYMV) (Fig. 1*A*). The retardation of mildew development is associated with the necrosis of leaf tissue in the areas of germinating conidia and the subsequent disintegration of the mildew (Fig. 1, *B* and *C*).

Leaves were excised from healthy and from virus-infected red clover inoculated with BYMV 6 weeks previously. The leaves were placed in petri dishes with the petioles submerged in 2-percent agar dissolved in water and were inoculated with the mildew fungus by brushing on conidia. After incubation for 48 hours (12 hours in darkness and 12 in artificial light) at 20° to 24°C under a 12-hour light period, germination of conidia was extensive on both virus-infected and virus-free tissue. Mycelial development was somewhat greater on virus-free than on infected tissue. On infected tissue a few microscopic necrotic areas were observed around some of the germinated conidia. Sixty hours after mildew inoculation, mycelial development was much more extensive on virus-free than on infected tissue. The necrotic areas around germinating conidia on infected tissue were present over the entire surface of the leaf. Necrosis of leaf tissue around areas of mildew development on virus-free clover was limited to a small area around the base of the leaflets, and in these areas development of the fungus was retarded. After 96 hours, mycelial development was uniform over almost all of the area of virus-free tissue, and there were several areas where conidiophores had developed. No conidiophores had developed on the virus-infected tissue, and no additional mycelial development had occurred. One hundred and fifty hours after mildew inoculation, the development of mycelium and conidiophores had increased in all areas of the virus-free tissue except near the bases of the leaflets. On virus-infected tissue the mildew development was almost completely inhibited and disintegration of the fungus was evident. The necrotic areas around the remnants of mildew were clearly visible to the naked eye.

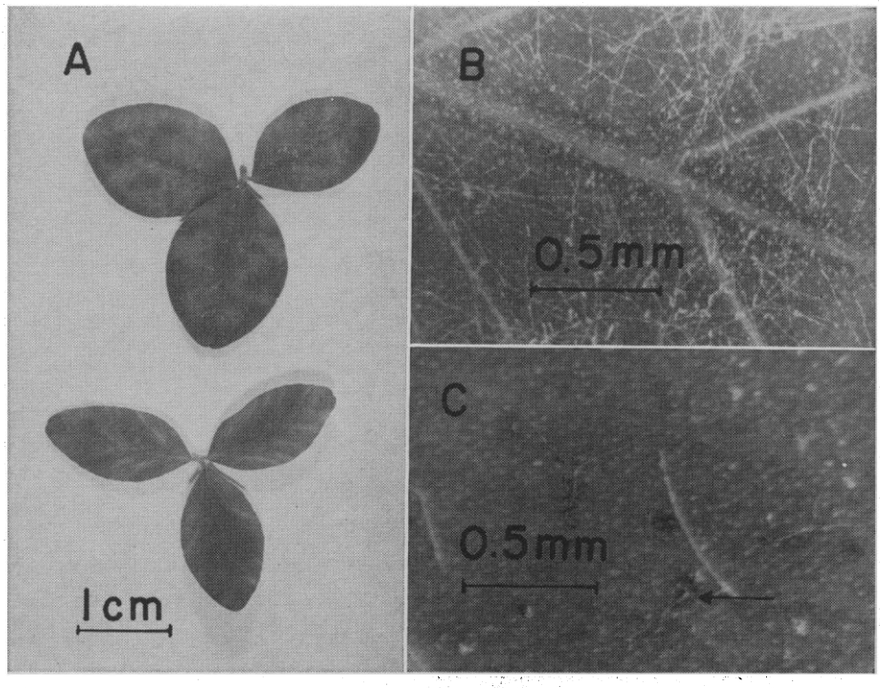

Fig. 1. Development of *E. polygoni* on Ky C 36 red clover. (*A*) The lower leaf was infected with BYMV; the upper leaf was virus-free. Both were inoculated with *E. polygoni*, which failed to develop on the virus-infected leaf. (*B*) Development of *E. polygoni* on virus-free leaf. The photographs in *B* and *C* were taken 7 days after inoculation of the leaves with *E. polygoni*. (*C*) Necrotic areas produced on virus-infected leaf tissue by inoculation with *E. polygoni*. Arrow indicates necrotic area around germinated conidium.

Similar patterns of mildew development were observed over a longer time period on virus-free and virus-infected clover in the greenhouse. Three weeks after mildew inoculation, luxuriant mildew development with abundant conidiophore production had occurred on virus-free tissue. A necrotic reaction of the host tissue and subsequent disintegration of the fungus was observed on virus-infected plants.

Smith (*4*) reported that mildew infection of highly resistant red clover was characterized by a rapid necrosis of tissue in the area of penetration of the fungus followed by disintegration of the fungus. The host plant which we employed (Ky C 36) is susceptible to powdery mildew when virus-free but becomes highly resistant when infected with BYMV after symp-

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,

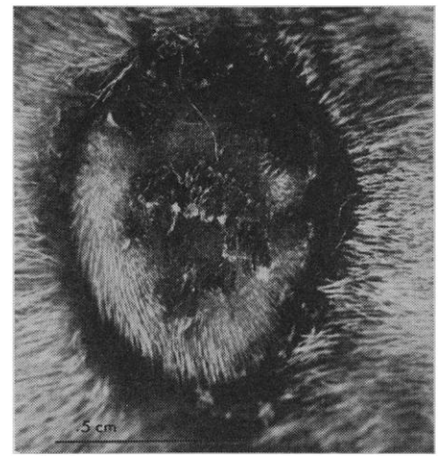

Fig. 1. Skin graft from Balb/c donor with virus induced leukemia on nonimmune Balb/c host after 14 days, showing areas of progressive necrosis.

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.