is the usual habit of the other species reared.

The *S. quadrifer* larvae are adjusted to, and dependent upon, the completion of their growth and metamorphosis in the stems of dodder hosts that they invade. Dissection of stems bearing dodder showed that after hatching in the dodder stem in June or July the young larvae had consumed most of the tissues in short sections of it, then penetrated the stem of the dodder host where they burrowed and fed until they had grown to their mature size by October (*10*). After chewing exit holes from their pupal cells to the surface and plugging the holes with excrement and detritus, they overwintered in the stems and pupated there in late April (in the field).

The adults are primarily associated with dodder. In the laboratory, they were strongly attracted to mature plants, seedlings, open seeds, or seed juices absorbed in paper, and they fed on the tender stem tips and buds. However, their egg-laying habits associate them with the dodder hosts that the larvae invade. Several females confined with males on dodder-infested *Solidago* plants in the laboratory deposited their eggs only in those portions of dodder stem that were directly attached to the stems of the hosts. When confined on loosely hanging portions of the dodder, the same females did not oviposit at all. In the field, mating pairs of *Smicronyx quadrifer* were found on dodder attached to *Vernonia noveboracensis*, *Artemisia vulgaris*, and *Solidago* sp., and the pattern of egg deposition associated with them was the same as that observed in the laboratory.

Female *Smicronyx sculpticollis* confined with males on dodder-infested *Solidago* plants laid very few eggs in the dodder stems. Only one living larva was recovered from the dodder stems, and no larval penetration could be detected in the *Solidago* stems. The fact that a few eggs were deposited in the dodder stems might, however, account for the occasional rearing of *Smicronyx sculpticollis* from hosts of dodder.

The factors underlying the development and maintenance of the host preferences shown by *S. quadrifer* will require further clarification. However, one apparent factor is the site of oviposition. Larvae hatching from eggs placed in the dodder stem near the haustoria are not only in position to enter the stem of the host of the dodder but may have to do so or perish, because the diameter of most dodder stems (0.8 to 2.0 mm in my material) is not large enough to accommodate mature larvae, which average 1.3 mm in body width, particularly after the dodder dies and shrinks with desiccation.

If the previously suggested evolution of host preferences (that is, from dodders to Compositae) in North American species of *Smicronyx* (*4*) is correct, the association described here may be significant as an intermediate stage in that sequence. There are apparently few recorded instances of a shift from one preferred host to another that are supported by well-documented intermediate stages (*11*).

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References and Notes

1. E. E. Gaertner, *Cornell Univ. Agr. Exp. Sta. Mem.* **294**, 3 (1950).
2. T. L. Harvey [*Ann. Entomol. Soc. Amer.* **59**, 1276 (1966)] studied aphid-dodder relationships and reported differences in aphid reaction to dodder growing on different hosts.
3. G. M. Baloch, A. I. Mohyuddin, M. A. Ghani, *Entomophaga* **12**, 481 (1967); *ibid.* **14**, 119 (1969).
4. D. M. Anderson, *Proc. U.S. Nat. Mus.* **113**, 185 (1962).
5. A. Hoffman, *Faune de France*, vol. 62, *Coléoptères Curculionides* (Lechevalier, Paris, 1958), p. 1412.
6. M. Q. Kahn and D. V. Murthy, *Indian J. Entomol.* **17**, 362 (1955).
7. The name *Smicronyx quadrifer* is applied in the broad sense here, because of my uncertainty of the taxonomic status of the eastern and western populations of the species.
8. The *Cuscuta* species were identified by Dr. E. E. Gaertner, Chalk River, Ontario; all other plant identifications were reviewed by Mr. C. V. Morton, Department of Botany, U.S. National Museum of Natural History.
9. R. M. Gilmer [*Phytopathol.* **48**, 432 (1958)] and other authors have described their use of dodder as a living bridge to transmit viruses from plant to plant.
10. Growth of larvae in stems of hosts of dodder can be demonstrated by measurement. Head widths of 34 young larvae dissected from dodder-infested stems of *Artemisia vulgaris* on 8 August 1969 varied from 0.20 to 0.42 mm, averaging 0.34 mm, whereas those of 47 mature larvae collected from the same situation on 18 October 1969 varied from 0.43 to 0.51 mm, averaging 0.47 mm.
11. V. G. Dethier, *Evolution* **8**, 33 (1954).
12. I thank Dr. V. G. Dethier (Princeton University) for a conference on the results of this study, and all others who gave advice or information while the work was in progress.
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Mixed Lymphocyte Cultures Produce Effector Cells: Model in vitro for Allograft Rejection

Abstract. *Mouse peripheral lymphocytes sensitized in vitro by culturing with allogeneic lymphocytes produced immunospecific destruction of target cells, as measured by release of chromium-51. Thus the sensitizing and effector phases of the cell-bound immune response can both be studied in an in vitro system.*

Immunity to allografts has been studied in vitro with the use of four independent test systems. The first is based on the proliferative response of nonimmune lymphoid cells when cultivated with allogeneic cells (the mixed lymphocyte interaction, MLI) (*1–3*) or with a cell-free preparation of the transplantation antigen (or antigens) (*4*). A second system is based on the destruction of target cells by lymphoid cells obtained from already immunized donors (*5, 6*). In a third type of test (*7*), target cells are cultivated with nonimmune allogeneic lymphocytes in the presence of phytohemagglutinin (PHA); this also results in the destruction of the target cells, presumably through a stage of lymphocyte blastoid transformation and proliferation initiated by the mitogen. In the fourth kind of test, the motility of macrophages is blocked by a combined action of antigen, sensitized lymphoid cell, and a factor released to the medium (*8*).

The immunologic specificity and selectivity of the proliferative phase of the MLI have been demonstrated (*2, 9*). It has been assumed that the reaction is directed against the (dissimilar) histocompatibility antigens of the stimulating lymphocyte population (*2, 3*) and thus represents in vitro the initial or "sensitizing" phase of the allograft immune response. If the production of effector cells could be demonstrated in these allogeneic lymphocyte cultures, this would give the final proof of the true immunologic nature of the MLI and make possible the study of allograft immune response in a genetically defined system in vitro. We tested this possibility, using mixed cultures of mouse peripheral leukocytes. In this test, the sensitizing phase of the immune response is characterized by incorporation of radioactive precursors into the replicating DNA of the responding lymphocyte population and the destructive phase by release of ^{51}Cr from labeled target cells undergoing cytolysis.

Under conditions already described

Table 1. Release of ^{51}Cr from L5178-Y ($H\text{-}2^d$) cells by lymphoid cells sensitized in vitro. The lymphocyte cultures were harvested on day 7. The incubation time was 6.5 hours.

Source of sensitized cells	H-2 allele combination	^{51}Cr release * (count/min)	Specific release (%)
C3H + C3D2F$_1$ †	k/k + k/d	4050	57.8
DBA/2 + C3D2F$_1$	d/d + k/d	1660	0.0
C3H + PHA-M (3 days)	k/k	1773	0.1
C3H	k/k	1735	0.0
DBA/2	d/d	1680	0.0
F$_1$ (C3H + DBA/2)	k/d	1745	0.0
	Medium from		
C3H + C3D2F$_1$		1662	0.0
DBA/2 + C3D2F$_1$		1734	0.0
(C3H + PHA-M)		1668	0.0
	Control		
Maximum release (freeze-thaw)		5756	100.0
Spontaneous release at 6.5 hours		1740	0.0

* Mean values of duplicate determinations. † F$_1$ (C3H + DBA/2).

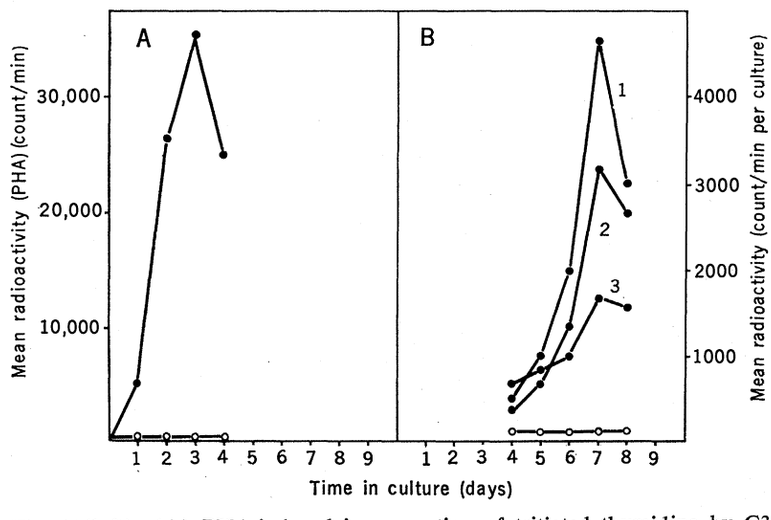
Table 2. Specific ^{51}Cr release by mouse peripheral lymphocytes sensitized in vitro with two sensitizing combinations and respective target cells. Lymphocyte cultures were harvested on day 6. Incubation time was 6 hours.

Sensitizing combination		^{51}Cr release (count/min)	
Strains	H-2	L5178-Y ($H\text{-}2^d$)	6C3H-ED ($H\text{-}2^k$)
C3H + C3D2F$_1$*	k/k + k/d	2228 (38.2%)	1682 (4.7%)
DBA/2 + C3D2F$_1$	d/d + k/d	657 (0.8%)	2609 (116.5%)
	Control		
Maximum release (freeze-thaw)		4734	2471
Spontaneous release (6 hours)		626	1643

* F$_1$ (C3H + DBA/2).

(*10*), peripheral leukocytes of C3D2F$_1$ animals (F$_1$ hybrids of C3H and DBA/2 strains) were cultivated together with equal numbers of leukocytes of either of the parental strains (C3H, $H\text{-}2^k$ or DBA/2, $H\text{-}2^d$) at a density of 1.5×10^6 lymphocytes per milliliter. Control cultures consisted of the same amount of lymphocytes from one donor only. The parental cells in mixed culture reacted against the alloantigens of the hybrid strain (*2*) by proliferation (one-way interaction) which could be measured by net incorporation of [^{3}H]thymidine or by autoradiography. The hybrid lymphocytes, on the other hand, are neutral toward both parental cell types. The peak point of the response in our test conditions was reached in culture at day 6 to day 7 (Fig. 1B) when, after 16 hours, up to 10 to 20 percent of the surviving cells in the mixed cultures were labeled. The nonmixed control cultures (Fig. 1B) did not respond to any significant degree. When the same amount of peripheral lymphocytes from any of the donor strains were cultivated in the presence of PHA (1:150 dilution, PHA-M, Difco Laboratories) an early vigorous proliferative response resulted (Fig. 1A). In contrast to the normal allogeneic response, the peak of the PHA-stimulated response was tenfold higher and was obtained as early as day 3 in culture.

To demonstrate the destructive phase, we cultivated leukocytes either in 40- to 50-ml quantities or in 1-ml quantities. The mixed (allogeneic) cultures were harvested on day 6 or 7, whereas the PHA-stimulated cultures were harvested on day 3. The lymphocytes sensitized in vitro were then incubated with ^{51}Cr-labeled target cells at a ratio of 60 to 100:1 (*6*). The target cells were labeled (*6*) with 0.1 mc of ^{51}Cr (sodium chromate, Amersham-Searle) just before the assay. These cultures, set up in duplicates in 13-mm Wassermann tubes, were harvested at intervals during 16-hour periods to determine the release of ^{51}Cr to the culture supernatant fluid.



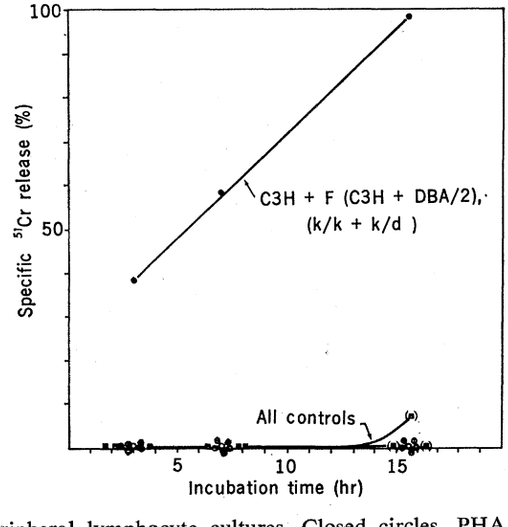


Fig. 1 (left). (A) PHA-induced incorporation of tritiated thymidine by C3H peripheral lymphocyte cultures. Closed circles, PHA present; open circles, controls. (B) Mixed lymphocyte interaction with C3H, DBA/2, and C3D2F$_1$ cultures. Curve 1, incorporation of [^{3}H]thymidine in (C3H + DBA/2); curve 2, in (C3H + C3D2F$_1$); curve 3, in (DBA/2 + C3D2F$_1$). Open circles: controls. Fig. 2 (right). Specific release of chromium from L5178-Y (DBA/2; $H\text{-}2^d$) cells by lymphocytes sensitized in vitro. Only the combination sensitized against $H\text{-}2^d$ causes specific release of the label. For controls, see Table 2.

In the first set of experiments, to test cytotoxicity, the following mixed lymphocyte cultures were prepared: (C3H + $C3D2F_1$) and (DBA/2 + $C3D2F_1$). The target was a mouse lymphoma line, L5178-Y, originating from DBA/2 ($H\text{-}2^d$) mice and carried both in vivo and in vitro (*11*). Controls consisted of (i) cultures containing cells derived from one donor only, (ii) PHA-stimulated cultures, which were collected on the third day, and (iii) supernatant culture fluid from all of the various cultures. As control for spontaneous ^{51}Cr release, target cells were incubated with fresh medium only; the maximum amount of label that can be released nonspecifically was determined by freezing and thawing a portion of labeled cells three times (Table 1). Only lymphocytes obtained from cultures specifically sensitized against antigens present in the target (C3H + $C3D2F_1$; $H\text{-}2^k$ + $H\text{-}2^{k/d}$) caused a specific release of the label. A significant proportion of the label was already released after 3 hours of incubation and reached 80 to 90 percent of the maximum release at 16 hours (Fig. 2). Cells obtained from the other mixed culture, sensitized against the other set of the hybrid's antigens (DBA/2 + $C3D2F_1$; $H\text{-}2^d + H\text{-}2^{k/d}$), or from PHA-sensitized lymphocytes were ineffective; nor did nonstimulated (nonmixed) cultures or any of the medium controls cause any specific release within the time of observation (Table 1).

To determine the specificity of the target cell, C3H and DBA/2 lymphocytes were similarly sensitized with $C3D2F_1$-hybrid cells in vitro and assayed against lymphoma cell lines originating from either of the parental strains, L5178-Y from DBA/2 and 6C3H-ED from C3H. The release of the ^{51}Cr in these experiments was equally specific and took place only when the sensitization in vitro in the lymphocyte cultures was directed against antigens present in the respective target cells (Table 2). In other words, the sensitized lymphocytes from the combination (DBA/2 + $C3D2F_1$; $H\text{-}2^d + H\text{-}2^{k/d}$) produced release of the label in a significant amount only from 6C3H-ED ($H\text{-}2^k$) cells and not from L5178-Y ($H\text{-}2^d$) cells or vice versa.

Although it has been shown in a xenogeneic system that rat lymphocytes cultivated on mouse fibroblast monolayer do become sensitized against the mouse cells and eventually kill them (*12*), this has not yet occurred in an allogeneic system without the use of a mitogenic agent, such as PHA (*7*). However, a specific sensitization in vitro of mouse lymph node cells by a transplantation antigen preparation results in (i) a specific cytotoxic interaction, as tested by the plaque-reduction assay (*11*), or (ii) in agglutination of sensitized lymphocytes around target cells (*13*). The mixed lymphocyte interaction is considered the initial or "sensitizing" phase of the allograft immune response in vitro. The essential immunologic requirements for the reaction, that is, selectivity and specificity, have also been demonstrated (*2, 9*). Although there was some preliminary evidence that the MLI would culminate in the production of "killer" lymphocytes (*14*), the immunologic specificity of the effector mechanism was not verified.

The destructive phase of the MLI is equally specific. Cytotoxic effect can be demonstrated only in cases where lymphocytes in mixed cultures are sensitized against antigens present in the target. The lack of effect of any of the medium controls, within the time of observation, also rules out nonspecific factors released to the medium (*15*).

In view of the specificity for both the afferent and the efferent responses, it is possible to consider the MLI a complete in vitro model for allograft rejection.

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References and Notes

1. K. Hirschhorn, F. Bach, R. L. Kolodny, I. L. Firschein, N. Hashem, *Science* **142**, 1185 (1963); B. Bain, M. Vas, L. Lowenstein, *Blood* **23**, 108 (19€ → R. W. Dutton, *J. Exp. Med.* **122**, 759 (1965).
2. D. B. Wilson, W. K. Silvers, P. C. Nowell, *J. Exp. Med.* **126**, 655 (1967).
→ F. H. Bach, H. Bock, K. Graupner, E. Day, H. Klostermann, *Proc. Nat. Acad. Sci. U.S.* **62**, 377 (1969).
4. L. A. Manson and T. Simmons, *Transpl. Proc.* **1**, 498 (1969).
5. W. Rosenau, in *Cell-Bound Antibodies*, B. Amos and H. Koprowski, Eds. (Wistar Institute Press, Philadelphia, 1963), p. 75; H. Friedman, *Science* **145**, 607 (1964); T. Vainio, O. Koskimies, P. Perlmann, H. Perlmann, G. Klein, *Nature* **204**, 453 (1964); D. B. Wilson, *J. Exp. Med.* **122**, 143 (1965); E. Möller, *ibid.*, p. 11; I. Hellström, *Int. J. Cancer* **2**, 65 (1967).
6. K. T. Brunner, J. Mauel, T. C. Cerottini, B. Chapuis, *Immunology* **14**, 181 (1968).
7. G. Holm, P. Perlmann, B. Werner, *Nature* **203**, 841 (1964).
8. S. Al-Askari, J. R. David, H. S. Lawrence, L. Thomas, *ibid.* **205**, 916 (1965).
9. M. R. Schwarz, *J. Exp. Med.* **127**, 879 (1968).
10. P. Häyry and V. Defendi, *Clin. Exp. Immunol.*, in press.
11. L. A. Manson, T. Simmons, L. Mills, H. Friedman, in *Proceedings of the Fourth Leukocyte Culture Conference*, D. R. McIntyre, Ed. (Appleton-Century-Crofts, New York, 1970).
12. H. Ginsburg, *Immunology* **14**, 621 (1968).
13. P. Koldolvsky, A. Turano, G. Fadda, *J. Cell. Physiol.* **74**, 31 (1969).
14. M. Virolainen, P. Häyry, V. Defendi, *Transplantation* **8**, 179 (1969).
15. T. W. Williams and G. A. Granger, *J. Immunol.* **102**, 911 (1969).
16. We thank S. I. Morse, Rockefeller University, for the pertussis supernatant; L. A. Manson for help with the manuscript, and for the L5178-Y cell line; Dr. G. Klein, Stockholm, Sweden, for the 6C3H-ED line; and Miss D. Rago for technical assistance. Supported by PHS contract 43-65-1028 from the Viral Carcinogenesis Branch, NCI, and by an American Cancer Society grant PRA-47. P.H. is the recipient of a travel grant from the National Medical Research Council, Helsinki, Finland.
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Mitochondrial RNA Synthesis during Mitosis

Abstract. *HeLa cells arrested in metaphase synthesized relatively normal amounts of mitochondrial RNA, while little RNA synthesis associated with the nucleus was detected. The RNA synthesized resembled the portion of mitochondrial RNA sensitive to ethidium bromide in interphase cells, with major peaks at 21, 12, and 4*S. *Unlike that in interphase cells, RNA synthesis in the mitochondrial fraction of mitotic cells was completely inhibited by ethidium bromide.*

The mitochondria of eukaryotic cells appear to be organelles possessing some degree of autonomy from the nucleus. In addition to DNA, both high molecular weight and 4*S* RNA, which is uniquely associated with the mitochondria of unicellular organisms, have been described (*1*).

The RNA specifically associated with mitochondria has been identified in animal cells. Cytoplasmic RNA which hybridizes with high efficiency to the circular DNA obtained from mitochondria has been described (*2*). Large molecular weight RNA associated with the mitochondria has been further characterized (*3–5*) and consists of two major species plus a large amount of heterogeneous RNA, as judged by acrylamide-gel electrophoresis. These two species have electrophoretic mobilities expected for RNA whose sedimentation constants would be 12 and 21*S* when compared to ribosomal RNA (*4*). The sedimentation velocity of the major species in sucrose-density gradients indicates a slightly different sedimentation constant than that predicted by electrophoretic analysis (*3, 4*). In lieu of a true sedimentation constant determination the values indicated by electrophoresis will