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18. The phytosaur ichnotaxon *Apatopus* occurs about 180 m below the Orange Mountain Basalt (N. Resch, personal communication) roughly 160 m below the estimated position of the Triassic-Jurassic boundary. The procolophonid reptile *Hypsognathus* [C. W. Gilmore, *Proc. U.S. Natl. Mus.* **73**, 1 (1928)] has been found roughly 120 m below the same basalt. Sedimentary cycles are not expressed in the areas which produced these remains, but by extrapolating from further southwest where cycles are present (without a change in total thickness of the formation) and under the rationale outlined in (*16*), these forms should be about 800,000 and 600,000 years older than the Triassic-Jurassic boundary, respectively.
19. Under close scrutiny the three apparent originations shown in Fig. 2 (Gephyrosauridae, Heterodontosauridae, and Megalosauridae) are questionable: either the stratigraphically oldest specimens assigned to the families are poorly dated or their taxonomic assignment is very doubtful.
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28. Another giant impact structure thought to be of early Mesozoic age is the Puchezh-Katuni structure in the Soviet Union which could be Early Triassic to Late Jurassic in age [V. I. Fel'dman, L. V. Sazonova, A. A. Nozova, *Int. Geol. Rev.* **27**, 68 (1985)].
29. The Triassic time scale of D. Webb [*J. Geol. Soc. Australia* **28**, 107 (1981)], with a Triassic-Jurassic boundary date of 200 million years ago, is used here. It agrees with the mean and modal K-Ar and $^{40}Ar/^{39}Ar$ dates for Newark Supergroup igneous rocks (based on a compilation by R. Hayden using corrected dates) and the U-Pb dates of D. L. Kimbrough and J. M. Mattinson [*Geol. Soc. Am. Abst. Prog.* **16**, 559 (1985), and personal communication]. However, other time scales give the boundary date as from 193 to 213 million years with uncertainties ranging from ±5 to ±15 million years. Jurassic time scale adapted from W. J. Kennedy and G. S. Odin [in *Numerical Dating in Stratigraphy*, G. S. Odin, Ed. (Wiley, New York, 1982), pp. 557–592] by fixing the Triassic-Jurassic boundary at 200 million years and scaling the position of the Hettangian-Sinemurian boundary by the number of ammonite zones. Other boundary dates are unaltered.
30. Geographic coordinates of localities are as follows: Blue Sac, 45°23′50″N to 45°24′15″N, 64°06′30″W to 64°10′00″W; Five Islands, 45°23′20″ to 40″N, 64°04′50″W; McKay head, 45°23′40″N, 64°13′00″ to 50″W; Wasson Bluff, 45°23′40″N, 64°14′00″ to 30″W.
31. We thank D. Baird, J. Bujak, B. Cornet, E. Robbins, and J. Shepard for sharing their unpublished data and interpretations; W. Alvarez, J. Andrews, M. Branden, T. Onstott, K. Padian, G. Pinna, D. Russell, and H.-D. Sues for helpful discussions; J. Andrews, C. Banach, N. Christie-Blick, S. Gould, R. Hayden, J. Hays, R. Schlische, and H.-D. Sues for criticizing preliminary drafts of the paper; W. Amaral, D. Baird, C. Banach, P. Chandoa, A. Heimlich, A. Litt, T. Haimov, A. McCune, J. Mercer, S. Orzak, L. Roth, P. Russ, R. Salvia, C. Schaff, H.-D. Sues for invaluable help in fossil collection; and W. Amaral, assisted by L. Davidson and J. Donham, for fossil preparation. Supported by a National Geographic Society research grant (3107-85), an ARCO Petroleum research fellowship, and a grant from the Alfred P. Sloan Foundation to P.E.O., a National Institutes of Health training grant, and support from the Barbor Fund of the Museum of Comparative Zoology and F. Jenkins to N. Shubin. Finally, we thank the Nova Scotia Museum, Halifax (especially R. Olgilvie and R. Grantham), and the Government of Canada for permission to collect and study in Nova Scotia and for research varied assistance. This paper is Lamont-Doherty Geological Observatory Contribution 4189.

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Organ-Resident, Nonlymphoid Cells Suppress Proliferation of Autoimmune T-Helper Lymphocytes

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Local presentation of autoantigen by organ-resident cells inappropriately expressing Ia determinants has been implicated in organ-specific autoimmunity. Experimental autoimmune uveoretinitis, induced in rats by immunization with retinal soluble antigen, is used as a model of organ-specific autoimmunity. In an in vitro system derived from this model, uveitogenic rat T-helper lymphocytes specific to the retinal soluble antigen, or control T-helper lymphocytes reactive to the purified protein derivative of tuberculin, were cocultured with Ia-expressing syngeneic retinal glial cells (Müller cells) in the presence of specific antigen. Antigen presentation was not apparent under ordinary culture conditions, and the Müller cells profoundly suppressed the proliferative response of primed T-helper lymphocytes to antigen presented on conventional antigen-presenting cells, as well as their subsequent interleukin-2 (IL-2)–dependent expansion. Suppression of proliferation was accompanied by inhibition of IL-2 production in response to antigen, as well as by reduction in high-affinity IL-2 receptor expression, and proceeded via a contact-dependent mechanism. These results suggest a role for locally acting suppression mechanisms in immune regulation and maintenance of tissue homeostasis.

IN ORGAN-SPECIFIC AUTOIMMUNE DISEASE the range of interactions between the component cells of a target tissue and the infiltrating autoreactive T cells is largely unknown. Recent observations have suggested active participation of organ-resident cells in tissue-specific autoimmunity. For example, vascular endothelial cells and brain glial cells (astrocytes) are able to express major histocompatibility complex (MHC) class II determinants (Ia) (the restricting elements of self-recognition by the immune system), to present antigen, and to produce interleukin-1 (IL-1)–like factors (*1, 2*). In fact, presentation of autoantigen by organ-resident cells aberrantly expressing Ia determinants was suggested as one of the major underlying causes in the induction and maintenance of the autoimmune state (*3*). Control of autoimmunity has been attributed to central suppression mechanisms (*4*); very little is known about locally acting mechanisms of suppression.

Experimental autoimmune uveoretinitis (EAU), like experimental allergic encephalomyelitis and adjuvant arthritis (*5*), serves as a model of organ-specific autoimmune disease and is mediated by T lymphocytes (*6, 7*). The disease can be induced in rats by immunization with the retinal soluble antigen (SAg) (a 48-kilodalton protein participating in light signal transduction), or by adoptive transfer of SAg-specific T-helper (T_H) lymphocytes (*6, 7*). EAU is basically a delayed-type hypersensitivity reaction to SAg and manifests itself as an ocular inflammation resulting in irreversible damage to photoreceptor cells, of which the SAg is a major constituent. Retinal glial cells (Müller cells)

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as well as other resident cells in the eye are capable of expressing Ia determinants (*8, 9*). Müller cells are a major structural component of the neural retina, closely associated with the photoreceptor cells and ensheathing the retinal blood vessels (*10*). Thus, they constitute the second barrier after the retinal vascular blood-brain barrier, which an infiltrating lymphocyte must pass in order to penetrate into the eye. We are able to grow essentially pure monolayer cultures of Müller cells from adult rat retinas in the presence of spleen concanavalin A (Con A) conditioned medium (SCM), which contains an unidentified growth factor for these cells. Characterization of these cultures by immunoperoxidase staining showed that they are positive for the glial cell marker glial fibrillary acidic protein, positive for the Müller cell marker (detectable with a monoclonal antibody developed in this laboratory), and contain about 30% Ia-positive cells (*8*).

In experiments designed to test the capacity of irradiated Müller cells to act as antigen-presenting cells (APCs) to a uveitogenic, SAg-specific T_H lymphocyte line (T_HS) (*6*), we noted that not only was antigen not presented, as judged by proliferation and measured by [^{3}H]thymidine incorporation of the T_HS cells, but even the background counts of the T_HS cells were inhibited. When we included Müller cells in the regular antigen stimulation system, containing rested T_HS, antigen and conventional APCs (irradiated syngeneic thymocytes), proliferation of the cells in response to antigen was inhibited by about 90% (Table 1). Suppression was effective even when the Müller cells were added 24 hours after the beginning of culture when maximal titers of IL-2 had already been produced by the T_HS cells in response to antigen. The inhibitory effect was not confined to SAg-specific cells because the T_HP control line, specific to the purified protein derivative of tuberculin (PPD), was inhibited to a similar extent (Table 1). The inhibition did not appear to involve a cytotoxic effect, since T_H cells recovered from the cocultures were fully viable.

Suppression of proliferation was accompanied by partial inhibition of IL-2 production in response to antigen and partial inhibition of IL-2 receptor generation by the T_H cells, as assayed by support of the growth of an IL-2–dependent cloned murine lymphocyte line (CTLL) by supernatants of suppressed cultures, and by binding of ^{125}I-labeled IL-2 (*11*) to the T_HS cells, respectively (Fig. 1, a and b). It is interesting that mainly the high-affinity form of the IL-2 receptor (detected at ^{125}I-labeled IL-2 concentrations of 0.1 n*M* or less), which is the functional form in terms of cellular proliferation (*12*), appeared to be affected (Fig. 1b). This may suggest that the interaction with the Müller cells was interfering with the assembly of the receptor into its high-affinity form (*13*), rather than with the synthesis of the receptor molecule.

The suppression did not appear to involve consumption of IL-2 or IL-1 by the Müller cells, since addition of excess lymphokine, in the form of rat SCM or recombinant human IL-2 and IL-1 (separately or in combination), did not abrogate or even diminish the suppression. Moreover, Müller cells do not absorb IL-2 activity from supernatants containing a known IL-2 titer and do not stain with monoclonal antibodies to the rat IL-2 receptor (*14*). Thus, the inhibition of IL-2 receptor generation may represent a primary phenomenon rather than a result of suboptimal lymphokine production in the system.

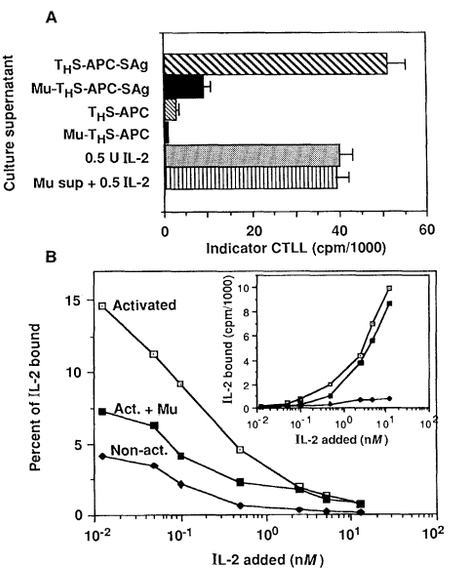
We hypothesized that the partial inhibition of antigen-driven IL-2 production and high-affinity IL-2 receptor generation could not fully account for the extent of suppression of T_HS cell proliferation and that there had to be an additional inhibitory effect acting later in the activation pathway. We could thus be dealing with a multistage suppression mechanism. This interpretation was further supported by the finding that

Table 1. Inhibitory effect of Müller cells on antigen-driven proliferation of SAg-specific (T_HS) and PPD-specific (T_HP) cells lines. Long-term cultures of T-helper lymphocytes (T_H) and Müller cells (Mu) were generated from Lewis rats and maintained as described (*6, 8*). The assays were carried out in a 1:1 mixture of the culture media from each of the two cell types [minimal essential medium with 10% fetal bovine serum and RPMI 1640, with 1.5% rat serum; each culture contained additional supplements as described (*6, 8*)]. Rested T_H cells (2×10^4) and APCs (syngeneic thymocytes irradiated with 3000 R) (4×10^5) were incubated with antigen in the presence or absence of 1×10^4 Müller cells (irradiated with 3000 R) in 96-well tissue culture trays (Costar), in triplicate 0.2-ml cultures. In the wells where coculture with Müller cells was to be delayed by 24 hours, the Müller and the T_HS cultures were plated separately at time 0, and the lymphocyte cultures were quantitatively transferred into the Müller cell wells at 24 hours of incubation. All cultures were incubated for 60 hours. One microcurie of [^{3}H]thymidine was added to each well during the last 14 hours. The background radioactivity of Müller cells alone was 0.4×10^{-3} cpm. Standard errors were less than 10% of the mean. ND, not done. Numbers in parentheses represent percent of inhibition.

Culture	[^{3}H]Thymidine uptake (cpm $\times 10^{-3}$)	
	T_HS	T_HP
Mu-T_H-APC-Ag*	3.5 (97.6)	7.9 (90.1)
Mu(24h)-T_H-APC-Ag†	18.3 (88.1)	ND
T_H-APC-Ag	153.5	80.1
Mu-T_H-APC	3.2	0.8
T_H-APC	2.7	0.5

*SAg or PPD (10 μg/ml) in T_HS and T_HP cultures, respectively. †Coculture with Müller cells started after 24 hours of activation with antigen and APCs.

Fig. 1. (**A**) Inhibition of IL-2 production and (**B**) inhibition of IL-2 receptor generation, in T_HS responding to antigen presented on conventional APCs, by Müller (Mu) cells in coculture. (A) Rested T_HS cells (2×10^4) and APCs (4×10^5) were incubated in the presence or absence of irradiated Müller cells (1×10^4) and of SAg (10 μg/ml) in triplicate 0.2-ml cultures, as for proliferation assays. The supernatants (sup) were collected at 24 hours and serial dilutions were assayed on triplicate 0.2-ml cultures of 5×10^3 CTLL cells, which were treated after 40 hours of incubation with 1 μCi of [^{3}H]thymidine per well for 8 hours. Shown are results obtained at 1:16 supernatant dilution ± standard errors. (B) T_HS at 2×10^5 cells per milliliter and APCs at 2.5×10^6 cells per milliliter were activated with SAg (5 μg/ml) on confluent or slightly subconfluent monolayers of Müller cells in 150-cm^2 culture flasks (Falcon) for 60 hours. The cells were recovered by shaking the flasks and were separated from APC debris by centrifugation over Ficoll (Isolymph, TEVA, Jerusalem) and washed. The cells were assayed for IL-2 receptor expression (and compared with T_HS activated in parallel without the presence of Müller cells and with nonactivated T_HS) by direct binding of ^{125}I-labeled IL-2 (New England Nuclear) essentially as described by Robb *et al.* (*11*), except that the last wash in oil was omitted. All dilutions of ^{125}I-labeled IL-2 were assayed in triplicate on 5×10^5 lymphocytes. Nonspecific background binding of ^{125}I-labeled IL-2 was determined under the same conditions on 5×10^6 sheep erythrocytes. The nonspecific binding in individual experiments was constant over the whole range of ^{125}I-labeled IL-2 dilutions, and was between 0.3% and 1.6% of the counts added (depending on the batch of ^{125}I-labeled IL-2). Results are expressed after background subtraction, as a plot of the percent of IL-2 bound versus the concentration of IL-2 added (nanomolar) for expansion of the high-affinity binding range, and as counts per minute of IL-2 bound versus concentration of IL-2 added for expansion of the low-affinity range (inset). Standard errors were less than, or equal to, 10% of the mean.



coculture with Müller cells was also effective in inhibiting fully activated T_HS cells, after they had already been exposed to antigen on APCs for 60 hours and required only IL-2 for continued proliferation. The activated T_HS cells were separated from APCs on a Ficoll density gradient, washed, and cultured for an additional 60 hours on monolayers of Müller cells in the presence of exogenously supplied IL-2. Under these conditions, proliferation of the T_HS cells was inhibited by about 50% compared to control T_HS, which were recultured in parallel without Müller cells. Paradoxically, addition of the specific antigen to this culture system resulted in a significant potentiation of the suppression, up to 80% compared to the control cells. Addition of an irrelevant antigen did not have this effect. High-affinity, but now low-affinity, IL-2 receptor expression was diminished in preactivated T_HS cells incubated on monolayers of Müller cells compared to the same cells cultured without Müller cells.

Having eliminated consumption of IL-2 by Müller cells as a possible suppression mechanism, we investigated whether the inhibition might be due to release of prostaglandins or leukotrienes, which are known to be suppressive in some systems (*15*). We tested the following inhibitors of arachidonic acid metabolism: indomethacin (inhibits cyclooxygenase and thereby prostaglandins), nordihydroguaiaretic acid (inhibits lipoxygenase and thereby leukotrienes) and *p*-bromophenacetyl bromide (inhibits phospholipase A_2, suppressing production of both leukotrienes and prostaglandins). None of these was able to reverse the inhibitory effect of Müller cells. We next tried to identify a putative inhibitory substance in supernatants of Müller cell cultures. However, addition of Müller cell supernatants or supernatants of cocultures of Müller cells and T_HS cells to cultures of T_HS cells reacting to antigen did not result in any detectable inhibition.

To differentiate between suppression by a labile inhibitory factor and suppression mediated by direct contact, we devised a one-step assay system using directly communicating cultures of Müller and T_HS cells. The method is based on plating the producer and the responder cultures in connected adjacent wells of a modified 96-well tissue culture tray, so as to allow free passage of the culture supernatant, but not of the cells, between the communicating wells (*16*). This method obviates the use of semipermeable membranes between the cultures, thus eliminating the possibility that a putative inhibitor might be binding to the membrane, and allows labeling and harvesting of replicate cultures in the usual way. The results of this assay clearly showed that the Müller and the T_HS cells must reside in the same well in order for the suppression to be effective (Table 2). Even in cultures in which one of the paired wells contained both T_HS and Müller cells and the other T_HS cells alone (with APCs and antigen added to both), the inhibition affected those T_HS cells that were in physical contact with the Müller cells; this result suggests that contact with the helper cells does not induce production of a soluble mediator of suppression. We believe that the contact-dependence of the inhibitory effect might explain the antigen-mediated potentiation of suppression exerted on the IL-2–supported growth of already activated T_HS cells: the T_HS cells may be binding to antigen displayed on the Müller cell surface, resulting in closer contact and thus facilitating inhibition. Increased clumping of Müller and T_HS cells, which occurred consistently in the presence of antigen, may support this explanation.

Long-term local presentation of autoantigen by organ-resident cells aberrantly expressing Ia determinants was thought to be one of the major underlying causes in the induction and maintenance of the autoimmune state (*3*). Expression of such determinants is readily induced on many organ-resident cells by γ-interferon not only in vitro but also in vivo (*17*). If any cell displaying MHC class II determinants is capable of antigen presentation, as has been suggested by the studies on Ia-expressing nonlymphoid cells and on L cells transfected with Ia genes (*1, 3, 18*), autoimmunity could be a potential sequel to any inflammatory situation where γ-interferon is produced. Our studies show that an organ-resident cell that expresses Ia determinants not only does not present antigen, but is actually capable of inhibiting antigen-induced activation as well as the subsequent IL-2–dependent expansion of primed autoimmune T_HS lymphocytes. In this context, it may be interesting that the inhibition appears to be independent of Ia expression by the Müller cells, since Müller cells that had been deprived of SCM and became Ia-negative were equally efficient in suppressing antigen-driven proliferation of T_HS. Suppression of IL-2–supported proliferation was not reversible by monoclonal antibodies to two different rat I-A determinants and an I-E determinant, at concentrations that inhibited Ia-restricted presentation of antigen by conventional APCs. Moreover, Müller cells were able to inhibit T cell proliferation across allogeneic and even across xenogeneic barriers (*14*). The eye has for a long time been considered an immunologically privileged site, exempt from certain immune reactions, similar to the brain and the hamster cheek pouch (*19*). This has been attributed to lack of lymphatic drainage of the interior of the globe. An intriguing question is whether cells such as the Müller cell might contribute to the phenomenon of immune privilege.

Müller cells are typically spared in various human ocular inflammatory conditions, and a commonly described sequel of ocular inflammation is the development of gliosis (*20*). In EAU the acute stage of the disease is followed by healing and scar formation, in which Müller cells appear to play an active role (*21*). We have previously shown that normal Müller cells from adult rat retinas are induced to proliferate in the presence of activated lymphocyte-monocyte products and that supernatants from T_H cells undergoing activation by antigen presented on syngeneic APCs are particularly effective in inducing this function (*5*). It is conceivable that these organ-resident cells, which are in close physical association with the photoreceptor cells that are the target of the autoimmune attack in EAU, may be activated by inflammatory cell products to proliferate and to act as a local suppression mechanism.

On the basis of the results reported here, we suggest that local immune reactions

Table 2. Requirement for close contact between T_HS cells and Müller (Mu) cells for effective inhibition of T_HS proliferation. The specified cultures were seeded in each of quadruplicate 0.2-ml connected wells, in a modified 96-well culture tray (*16*). The || symbol denotes the communicating cultures. Each 0.2-ml well contained 2×10^4 T_HS ($+ 4 \times 10^6$ APCs) and 1×10^4 Müller cells (irradiated with 3000 R), individually or in coculture. After the cells were allowed to settle for 2 hours, supernatants of the paired cultures were brought into contact and the antigen was added in a volume of 10 μl to the specified cultures, to the final concentration of 10 μg/ml. Culture medium was as described in Table 1. The cultures were incubated for 60 hours and were exposed to 1 μCi of [^{3}H]thymidine per well for the last 14 hours. Standard errors were less than 10% of the mean.

Culture	[^{3}H]Thymidine uptake (cpm × 10^{-3})	
	S-Antigen added	No stimulant
None || T_HS-APC	59.3	0.1
Mu || T_HS-APC	3.9 44.5	3.7 0.1
None || Mu-T_HS-APC	5.3	3.8
T_HS-APC || Mu-T_HS-APC	42.8 3.3	0.1 3.6

might in some cases be controlled by mechanisms not involving suppressor T cells. Such mechanisms could play a role in protecting the integrity of organ structures from autoimmune attack, or from bystander damage during local inflammatory reactions, by curbing in situ the activation and clonal expansion of immune T lymphocytes.

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Tolerance Induced by Thymic Epithelial Grafts in Birds

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Grafts of the anterior limb bud introduced at embryonic day 4 between histoincompatible chick embryos were subject to chronic, mild rejection beginning from several weeks to several months after birth. In contrast, quail wing buds similarly grafted into chickens started to be rejected at the first or second week after birth and finally autoamputated. Embryonic thymus epithelium from donor quail (before it had been colonized by hemopoietic cells) was grafted into chicks. A chimeric thymic epithelial stroma was generated in which the lymphocytes of the chick acquired the capacity to recognize the grafted limb as self either permanently or for a protracted period of time. In such thymic chimeras the grafted wings were not rejected.

SELF AND NONSELF RECOGNITION IS acquired during development and is then maintained throughout life. The thymus plays a central role in immunological recognition of self and nonself. T lymphocytes that have differentiated in the thymus become able to recognize, through a surface receptor, foreign antigens in association with molecules encoded by the major histocompatibility complex (MHC) (*1–4*).

Although the role of intrathymic differentiation is important in MHC restriction of T cells, the selection process of the T cell repertoire in the thymus is not understood. In particular, the respective roles of the stable endodermal component of the thymic stroma and of the macrophages and dendritic cells of hemopoietic origin are not known. Both cell types express class I and class II MHC antigens from early embryonic stages onward in mammals (*5*) and birds (*6*).

Attempts have been made to induce allotolerance in the adult mouse by grafting fetal thymuses depleted of their lymphocytes by deoxyguanosine or by culture at low temperature; these experiments have yielded controversial results. The grafted thymus, although expressing donor MHC antigens, survives for a long time in an allogeneic environment; nevertheless, it fails to induce allotolerance as tested in mixed lymphocyte reactions (MLR) when grafted into a normal histoincompatible recipient (*7*). However, thymuses cultured at low temperature before grafting, introduced into an athymic nude mouse, induce tolerance, as judged by MLR with both intrathymic and spleen cells (*8*). Thymic epithelium grafts (after deoxyguanosine treatment) can tolerize precursors of cytolytic T cells, but only for the minor and not the major histocompatibility antigens carried by the thymic epithelial cells (*9*). In fact, none of these experiments resolves if the induction of tolerance to self-MHC products is mediated through the thymic epithelium itself or by remaining cells of the dendritic and macrophage lineages, because neither method completely eliminates the latter category of cells.

We have investigated the establishment of tissue tolerance during ontogeny to see to what extent early embryonic grafting of allogeneic tissues leads to tolerance when the host's immune system is exposed to foreign antigens during development (that is, in the embryo itself). We grafted limb buds at day 4 of embryonic development (E4) between chick embryos of different histoincompatible haplotypes, and the embryos were allowed to hatch.

The allogeneic wing grew normally and did not show signs of rejection for a period of time varying from 1 to 5 months. Such signs eventually always appeared, however, showing that partial, but not complete, tolerance of the graft could be induced by early embryonic grafting experiments.

We then devised a system in which rejection of the embryonic graft takes place acutely as soon as the young chicken's immune system becomes mature (that is, during the second week after hatching). This was achieved in 100% of cases by xenogeneic grafting of quail embryonic limb buds onto chick recipients under conditions similar to those for allogeneic transplantations. In this model, grafts of quail thymic epithelium introduced before seeding of the epithelium by hemopoietic cells induced tolerance of the foreign limb.

The grafts were carried out as in Fig. 1a. Some of the chickens used in these experiments were partly inbred strains of the B14 and B19 genotypes; in other experiments they were F1 embryos of the inbred strains B15 × B21 (*10*). Fourteen chimeras hatched out of 114 grafted embryos. In most cases, the grafted wing was well formed and mobile. In others, the wing was somewhat shortened. Further development

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