hits (88% correct), and misses (89% correct) ($F < 1.0$). Semantic decision RTs declined across trial types [$F(2,24) = 9.26$]: RTs were longer for high confidence hits (1000 ms) compared to low confidence hits (966 ms) [$F(1,12) = 5.40$], which were in turn longer compared to misses (936 ms) [$F(1,12) = 3.91$, $P < 0.06$].

16. The procedures for selective averaging and statistical map generation for rapidly intermixed trials are described elsewhere (7). Statistical activation maps were constructed based on the differences between trial types using a *t*-statistic (7). Fixation trial events were subtracted from the word trial events (collapsing across subsequent memory). Miss trial events and high confidence hit events were subtracted from each other, as were those for miss trial events and low confidence hit events. Clusters of five or more voxels exceeding a statistical threshold of $P < 0.001$ were considered significant foci of activation (7).
17. In addition, modest but significantly greater activation for high confidence hits relative to misses was noted in a more ventral extent of left inferior frontal gyrus [Brodmann's area (BA) 47: −34, 31, −3], left precentral gyrus (BA 6: −31, 0, 56), medial superior frontal gyrus (BA 8: −3, 28, 43), and left superior occipital gyrus (BA 19: −31, −77, 34). The superior occipital and medial superior frontal activations can be seen in Fig. 2. Two regions demonstrated less activation for high confidence hits relative to misses: precuneus (BA 31: 3, −43, 40) and left middle frontal gyrus (BA 9: −12, 31, 34). No regions demonstrated greater activation for low confidence hits relative to misses, which is in accord with the behavioral data indicating that these two trial types likely did not mnemonically differ.
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19. RTs were matched as follows. First, the median RT across all trial types was determined for each subject. Trials with response latencies that fell below the median RT were selected and sorted based on subsequent memory. Selection of trials in this manner resulted in matched RTs for the high confidence hit (852 ms) and miss (839 ms) trial types [$F(1,12) = 2.32$, $P > 0.15$)].
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25. Supported by grants from the National Institute on Aging (AG08441 and AG05778), the National Institute on Deafness and Other Communication Disorders (DC03245-02), the Human Frontiers Science Program, and the Deutsche Forschungsgemeinschaft (SFB426). We thank Y. and W. Jacobson and two anonymous referees for helpful comments on an earlier version of this manuscript, and C. Brenner and C. Racine for assistance with data collection.

15 May 1998; accepted 20 July 1998

Prevention of Allogeneic Fetal Rejection by Tryptophan Catabolism

David H. Munn, Min Zhou, John T. Attwood, Igor Bondarev, Simon J. Conway, Brendan Marshall, Corrie Brown, Andrew L. Mellor*

In 1953 Medawar pointed out that survival of the genetically disparate (allogeneic) mammalian conceptus contradicts the laws of tissue transplantation. Rapid T cell–induced rejection of all allogeneic concepti occurred when pregnant mice were treated with a pharmacologic inhibitor of indoleamine 2,3-dioxygenase (IDO), a tryptophan-catabolizing enzyme expressed by trophoblasts and macrophages. Thus, by catabolizing tryptophan, the mammalian conceptus suppresses T cell activity and defends itself against rejection.

Medawar (*1*) considered three mechanisms that might explain the immunological paradox of fetal survival: (i) anatomic separation of mother and fetus, (ii) antigenic immaturity of the fetus, and (iii) immunologic "inertness" (tolerance) of the mother. In view of evidence that the entire repertoire of maternal T cells specific for paternally inherited major histocompatibility complex (MHC) class I alloantigens is transiently affected and tolerized during pregnancy (*2, 3*), the first two mechanisms cannot explain fetal allograft survival, and attention has focused on the third mechanism. Certain macrophages, induced to express IDO in response to interferon-γ and other signals from activating T cells, inhibit T cell proliferation in vitro by rapidly consuming tryptophan (*4, 5*); some tissue macrophages may use this immunosuppressive mechanism in vivo. Because IDO is also expressed by human syncytiotrophoblast cells (*6*) and systemic tryptophan concentration falls during normal pregnancy (*7*), we formulated the hypothesis that IDO expression at the maternal-fetal interface is necessary to prevent immunological rejection of fetal allografts. To test this hypothesis, we exposed pregnant mice (carrying syngeneic or allogeneic fetuses) to 1-methyl-tryptophan, a pharmacologic agent that inhibits IDO enzyme activity (*8*).

First, IDO transcription during pregnancy (*9*) was assessed in females mated with CBA (syngeneic) or C57BL/6 (B6, allogeneic) males (Fig. 1). IDO transcripts were detected from 7.5 to 9.5 days post coitus (dpc) in all concepti but were not detected at 6.5 dpc. At later gestation times (10.5 and 13.5 dpc), IDO transcripts were detected in placenta but not in maternal uterus or embryonic tissues. These findings are consistent with the known expression of IDO in human syncytiotrophoblast (*6*).

Pregnant mice ($n = 8$ to 32) carrying syngeneic or allogeneic concepti were treated with 1-methyl-tryptophan or with placebo, beginning at 4.5 dpc (*10*). Concepti were examined macroscopically and histologically at various times during gestation (*11*). At 6.5 dpc, mice from all treatment groups carried normal numbers of concepti and embryonic development was normal (Table 1). At 7.5 to 8.5 dpc, the mean number of allogeneic concepti in females treated with IDO inhibitor was reduced significantly ($P < 0.01$) and extensive hemorrhaging surrounded most of those that remained (Fig. 2A). However, at 7.5 dpc, most remaining allogeneic concepti were developmentally normal (Fig. 2C), with rare embryos showing signs of degeneration. By 8.5 (Fig. 2F) to 9.5 dpc, all allogeneic embryos showed signs of inflammation and progressive deterioration (*12*). After 9.5 dpc, no allogeneic concepti remained in any mice treated with IDO inhibitor. In contrast, the mean number of syngeneic concepti and the developmental status of embryos were not af-

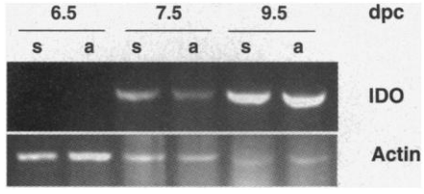

Fig. 1. Analysis of IDO transcription during murine gestation. PCR products were generated by RT-PCR amplification from RNA samples prepared from pooled syngeneic (s) or allogeneic (a) concepti, at the gestation times indicated (9). Results are representative of three separate experiments.

D. H. Munn, M. Zhou, J. T. Attwood, I. Bondarev, S. J. Conway, B. Marshall, A. L. Mellor, Programs in Molecular Immunology and Developmental Biology, Institute of Molecular Medicine and Genetics, and Department of Pediatrics, Medical College of Georgia (MCG), 1120 15th Street, Augusta, GA 30912, USA. C. Brown, Department of Pathology, University of Georgia, Athens, GA 30602, USA.

*To whom correspondence should be addressed. E-mail: mellor@immag.mcg.edu

fected by exposure to 1-methyl-tryptophan during the period 6.5 to 15.5 dpc (Table 1 and Fig. 2). No evidence of injury or inflammation to syngeneic concepti resulting from exposure to 1-methyl-tryptophan was observed upon histological examination (Fig. 2, B and E).

Table 1. Effect of inhibition of IDO activity on rejection of allogeneic concepti (*10*).

Mating genotype (♀ × ♂)	Gestation stage (dpc)	Mean no. concepti per ♀ (no. ♀ treated)		Appearance of concepti	
		Inhibitor	Placebo	Inhibitor	Placebo
CBA × CBA	6.5–8.5	7.3 (3)	7.5 (2)	Normal	Normal
	9.5–15.5	6.7 (7)	6.5 (6)	Normal	Normal
CBA × B6	6.5	7.3 (3)	8.5 (2)	Normal	Normal
	7.5–8.5	5.5* (10)	8.3 (6)	Majority inflamed	Normal
	9.5–15.5	0.2** (19)	5.4 (9)	Absent	Normal

*$P < 0.01$ or **$P < 0.0001$ by analysis of variance compared to placebo controls at each time point.

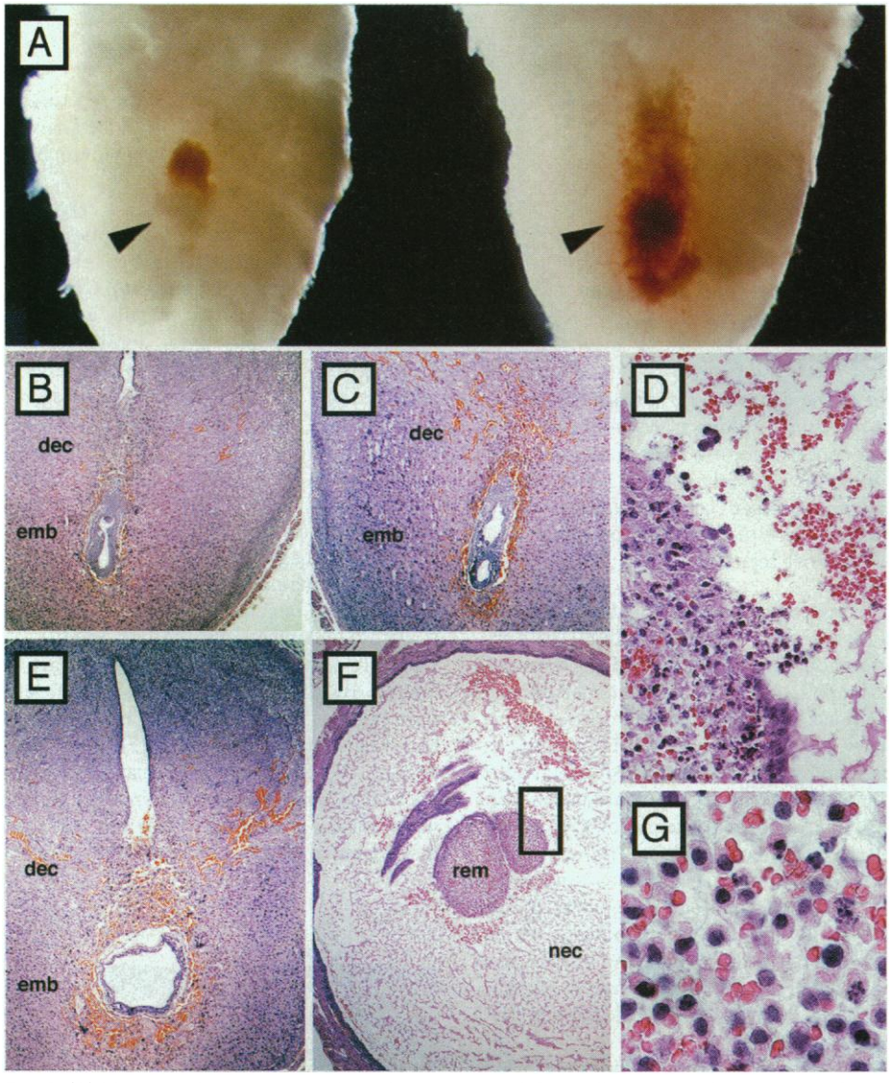

Fig. 2. (**A**) Macroscopic appearance of 7.5 dpc syngeneic (left) or allogeneic (right) conceptus from mice treated with 1-methyl-tryptophan (*10*). Arrows indicate the location of embryos. Extensive hemorrhaging surrounds the allogeneic conceptus. (**B** to **G**) Histology of syngeneic conceptus [(B) and (E)] and allogeneic conceptus [(C) and (F)] from pregnant mice treated with IDO inhibitor at 7.5 dpc [(B) and (C)] or 8.5 dpc [(D) to (G)]; (D) is an enlargement of the boxed area in (F), and (G) gives detail of area in lower left of (D) showing mononuclear cell infiltrate. Key: dec, decidua; emb, embryo; rem, remnant of resorbing decidua; nec, necrotic tissue. Results are representative of examinations of eight concepti. Magnifications: ×40 [(A) to (C) and (E) and (F)], ×600 (D), and ×1000 (G).

The contribution of maternal lymphocytes to induced loss of allogeneic concepti was assessed using females carrying a defective *RAG-1* gene (*RAG-1*$^{-/-}$), which prevents lymphocyte development (*13*). Female *RAG-1*$^{-/-}$ mice (with CBA backgrounds) were mated with B6 males and treated with 1-methyl-tryptophan as before. All females examined at 11.5 dpc had normal numbers of healthy concepti (Table 2). *RAG-1*$^{-/-}$ females treated with 1-methyl-tryptophan carried to term, delivering healthy litters of normal size. However, pregnant *RAG-1*$^{-/-}$ mice mated with B6 males lost all their allogeneic concepti before 12.5 dpc when reconstituted with a defined population of H-2K^{b}–specific T cells from BM3 T cell receptor (TCR) transgenic mice (Table 2) (*14*). Thus, maternal lymphocytes are essential for rejection of allogeneic concepti to occur in mice exposed to 1-methyl-tryptophan.

To test whether a single paternally inherited MHC class I difference provokes fetal rejection, we mated CBA females with CBA males (GK) carrying a recombinant H-2K^{b} transgene expressed under the control of promoter elements from the human *HLA-G* gene (*3*). The GK transgene drives H-2K^{b} expression in trophoblast from early gestational times but has no effect on fecundity (Table 2). However, CBA females mated with GK male mice and exposed to 1-methyl-tryptophan lost all their concepti before 11.5 dpc (Table 2).

These data show that inhibition of tryptophan catabolism during pregnancy allows maternal lymphocytes to mediate fetal rejection. This outcome was predicted from our hypothesis that placental cells expressing

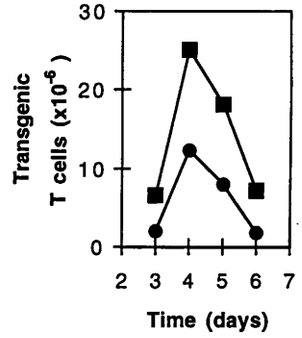

Fig. 3. Effect of 1-methyl-tryptophan on T cell responses elicited after adoptive transfer of H-2K^{b}–specific CD8^{+} T cells into CBA × B6 (F_1) recipient mice. Splenocytes (10^7) from BM3 TCR transgenic mice were injected intravenously on day 0 (*14*). Groups of mice received either 1-methyl-tryptophan (squares) or placebo control (circles) at the time of adoptive transfer (*10*). Two recipients were analyzed on each day. The mean number of donor CD8^{+} T cells in spleen was assessed by flow cytometry using an anticlonotypic monoclonal antibody (*14*). Results are representative of five separate experiments in which a total of 23 recipients treated with 1-methyl-tryptophan and 23 controls were examined.

Table 2. Effect of maternal lymphocytes and one MHC class I alloantigen on embryo rejection (*10*).

Mating genotype (♀ × ♂)	IDO inhibitor	No. ♀ examined (dpc)	Mean no. concepti per ♀
CBA (*RAG-1*$^{-/-}$) × B6	Yes	5 (11.5)	7.8
CBA (*RAG-1*$^{-/-}$) × B6	Yes	2 (at term)	7 (pups/litter)
BM3* → CBA (*RAG-1*$^{-/-}$) × B6	Yes	4 (12.5–14.5)	0
CBA × GK†	Yes	4 (11.5)	0
CBA × GK†	No	21 (at term)	6.4 (pups/litter)

*CD8$^+$ T cells in BM3 TCR transgenic mice recognize H-2K^b (*14*). †GK mice are H-2K^b transgenic mice made on the CBA background (*3*).

IDO suppress T cell proliferation. Most likely, maternal T cells induce fetal loss, because rejection (i) was triggered by H-2K^b, which induces potent T cell responses; (ii) occurred 4 to 5 days after implantation (4.5 dpc), making it unlikely that B cells could produce antibodies in time; and (iii) occurred when pregnant *RAG-1*$^{-/-}$ mice were reconstituted with H-2K^b–specific T cells from TCR transgenic mice. Additional evidence that T cell responses are augmented when mice are exposed to 1-methyl-tryptophan was obtained by monitoring T cell responses elicited after adoptive transfer of H-2K^b–specific T cells into recipients expressing H-2K^b (*14, 15*). Increased numbers of activated donor T cells were recovered from spleens of recipient mice (CBA × B6) treated with 1-methyl-tryptophan (Fig. 3), showing that alloreactive T cell responses had been partially suppressed by cells catabolizing tryptophan. We conclude that fetal rejection is initiated by maternal T cells that are normally suppressed by cells catabolizing tryptophan. Which T cell–dependent effector cells (for example, cytotoxic T cells, activated macrophages) ultimately destroy allogeneic concepti remains to be determined.

The possibility that fetal loss was caused by nonspecific toxic effects of 1-methyl-tryptophan was eliminated because the inhibitor had no effect on syngeneic pregnancies relative to placebo controls. Moreover, the requirement for maternal lymphocytes demonstrates that 1-methyl-tryptophan is not itself abortifacient. This study was prompted by our observation that certain macrophages inducibly expressing IDO inhibit T cell proliferation in vitro by catabolizing tryptophan (*5*). 1-Methyl-tryptophan was selected because it is a known inhibitor of IDO (*8*), enabling us to test our hypothesis. For these reasons it is likely that IDO is the relevant target of the inhibitor in vivo. Although we cannot formally rule out that another target exists, it seems unlikely that there is a second enzyme that regulates T cell responses and is inhibited by 1-methyl-tryptophan.

In human placenta, IDO is expressed by fetal-derived syncytiotrophoblasts at the maternal-fetal interface (*6*). We do not yet know the identity of cells expressing IDO in murine trophoblasts. On the basis of the human data, we predict that IDO-expressing cells of fetal origin in murine trophoblasts will tolerize maternal T cells in situ. However, it is possible that fetal cells may migrate and perform immunoregulatory functions at other maternal sites, as has been described (*16*).

The differentiation and growth factor MCSF (macrophage colony-stimulating factor) was used to generate macrophages that suppress in vitro T cell proliferation by catabolizing tryptophan (*5*). MCSF is also an important growth factor in trophoblast (*17*). We speculate that MCSF may influence the differentiation of cells capable of expressing IDO and of suppressing T cell proliferation in trophoblast and other normal tissues.

Our in vitro studies on immunosuppressive macrophages (*5*) and the current study provide a mechanistic link between tryptophan catabolism by cells expressing IDO and induction of maternal T cell tolerance to fetal allografts. We do not know precisely how T cell tolerance is achieved. Cells at the maternal-fetal interface (or in maternal lymphoid tissues) expressing IDO may establish local microenvironments in which reduced tryptophan concentration precludes T cell proliferation. Transplacental transport of tryptophan may assist this process. We have seen no evidence of an immunosuppressive metabolite produced by tryptophan catabolism (*18*), but this is an alternative possibility.

We conclude that the mammalian trophoblast (placenta) is not an anatomical barrier between maternal lymphocytes and the fetus; nor is it antigenically immature, because potent maternal T cell responses are elicited if tryptophan catabolism is inhibited. In this respect, the allogeneic conceptus is like all other allogeneic tissue grafts. The special characteristic that distinguishes the trophoblast from other tissues is its ability to suppress maternal T cell responses. Thus, we propose another mechanism to add to Medawar's original list: The fetal allograft actively defends itself from attack by maternal T cells.

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9. Female CBA mice were mated with syngeneic or allogeneic (B6) male mice. Females with vaginal plugs (0.5 dpc) were examined at times indicated. Total RNA was prepared from dissected conceptus by homogenization in RNA-STAT 60 solution (Tel-TestB Inc.). Transcripts of the murine IDO gene [see (*19*)] were detected by the reverse transcription polymerase chain reaction (RT-PCR) using forward (GTACATCACCATGGCGTATG) and reverse (GCTTTCGTCAAGTCTTCATTG) oligonucleotide primers. PCR products were of the expected size (740 bp). RT-PCR conditions used were 48°C for 45 min, 94°C for 2 min (1 cycle); 94°C for 30 s, 58°C for 1 min, 68°C for 2 min (40 cycles); and 68°C for 5 min (1 cycle). PCR products were fractionated on a 1.5% agarose–TBE gel containing ethidium bromide and were visualized by ultraviolet fluorescence. RT-PCR amplification of the murine α-actin gene (480 bp) was performed in parallel.
10. Slow-release polymer pellets impregnated with 1-methyl-tryptophan (0.9 mg/hour) or placebo pellets were inserted surgically under dorsal skin at 4.5 dpc. Pregnant mice were examined at gestation times indicated. Results are summarized in Tables 1 and 2. Fecundity rates for mouse colonies bred at our institution are 6.4 (CBA × CBA and CBA × GK) and 5.4 (CBA × B6) pups per litter at parturition. All procedures involving mice were conducted in strict accordance with institutional guidelines for animal care.
11. Tissues were prepared for sectioning by fixing them in 4% paraformaldehyde. Serial sections (5 μm) were prepared using a microtome and were stained with hematoxylin and eosin before microscopic examination.
12. During rejection, we observed mixed mononuclear cell infiltrates, disruption of trophoblast, and deterioration of the developing embryo. After rejection, all that remained were remnants of decidua surrounded by inflammatory infiltrates, necrotic tissue, and cellular debris.
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20. We thank A. Wylds, D. McCool, and R. Rogers for technical assistance; E. Simpson, S. Rastan, and S. Goldman for critiquing; and A. Compton for preparing the manuscript. Supported by the MCG Research Institute (A.L.M. and S.J.C.), NIH awards (D.H.M. and S.J.C.), the March of Dimes (S.J.C.), the MCG Department of Medicine, and the Carlos and Marguerite Mason Trust.

27 April 1998; accepted 7 July 1998