no apparent injury to the transplanted kidneys.

Perhaps in our study the failure of passive immunization alone, to prevent completely rejection of the graft, may be due to the absence of antibody directed against strain-specific renal antigens. It has been shown that maximum suppression of rejection of tumor allografts requires antibody against all antigens present in the donor and absent from the host (*13*).

Antibodies against donor antigens were prepared in animals syngeneic to the recipients. Therefore, in this model system, the antiserum used for passive immunization is thought to contain antibodies against all histocompatibility antigens present in the donor and absent from the recipient. In genetically diverse populations difficulty is apparent in obtaining a single antiserum reacting specifically with all allograft histocompatibility antigens that the recipient lacks. Use of antiserum from multiple donors may provide a sufficiently wide spectrum of antibody activity. Alternatively it may be possible to immunize the recipient with donor antigen so that antibody is produced but delayed hypersensitivity does not develop; in this way the recipient could acquire antibody capable of reacting against all graft antigens.

The broadly nonspecific immunosuppressive agents in current clinical use depress the host's ability to react to all antigens, and impair his defenses against ubiquitous pathogens; consequently infection, not rejection, is the most frequent cause of death among recipients of transplants. The treatment with antigen and antiserum combined suggests a means for specific suppression of graft rejection after transplantation of organs.

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Hypersensitivity: Specific Immunologic Suppression of the Delayed Type

Abstract. *Delayed-type cutaneous hypersensitivity to sheep erythrocytes was induced in rats by intradermal injection of the antigen mixed with Freund's adjuvant; hypersensitivity was sustained by weekly injections. Either passive immunization with rat antiserum to sheep erythrocytes or intravenous injection of sheep erythrocytes partially suppressed induction of hypersensitivity; these procedures used together specifically and completely suppressed induction of hypersensitivity. Complete suppression was sustained by antigen given intravenously before each weekly injection of the mixture of antigen and adjuvant. These findings provide the rational basis of a simple method for prolonging survival of allografts with only the biological agents, antigen and antibody, of the immunological response.*

Immunological reactions to an antigen divide into two broad categories: (i) antibody and (ii) cell-mediated (delayed-type hypersensitivity) responses. Either or both responses may be induced, depending on immunization procedures. Selective, specific suppression of the antibody or the cell-mediated response may be desirable for special purposes. For example, the antibody response of animals not previously exposed to an antigen can be specifically and profoundly suppressed by passive immunization with antibody to the antigen (*1*). Advantage is taken of this phenomenon to prevent immunization of Rh-negative mothers by fetal Rh-positive erythrocytes (*2*). On the other hand, a cell-mediated rather than an antibody response probably mediates rejection of allografts (*3*). We now report use of antigen and antibody together to suppress specifically induction of delayed-type hypersensitivity in the rat.

Adult female Sprague-Dawley rats were sensitized to sheep erythrocytes (SRBC). Emulsion (0.5 ml) consisting of equal parts of 5 percent SRBC in saline and of Freund's complete adjuvant (FCA) was injected intradermally in three depots in abdominal skin. For demonstration of hypersensitivity, 0.1 ml of a 7.5-percent suspension of SRBC in saline was injected intradermally on the dorsum of a hind paw; the extent of swelling provided a measure of hypersensitivity; measurements could be made rapidly, accurately, and repeatedly by weighing of the volume of mercury displaced by the paw (*4*). Hypersensitivity was most severe 9 to 11 days after sensitization; swelling of the paw began after about 12 hours and became most severe 18 to 24 hours after challenge. In the following experiments rats were challenged 9 days after sensitization, and responses were measured 21 hours later. By multiple criteria the challenge reaction was completely analogous to a cell-mediated, delayed-type hypersensitive reaction previously described for the rat (*4*, *5*). A high degree of cutaneous hypersensitivity to SRBC could be maintained by weekly intradermal injections of SRBC with FCA; weekly injections of either antigen or adjuvant alone did not maintain hypersensitivity.

Development of hypersensitivity was reduced by either of two procedures: (i) intravenous injection of antigen, or (ii) passive immunization. Rats were injected intravenously with 1.0 ml of 5 percent SRBC in saline 1 day before sensitization by SRBC with FCA. Challenge responses were reduced in severity by about 60 percent. Other rats were passively immunized with a total of 4.0 ml of hyperimmune-rat antiserum to SRBC given intravenously in 1.0-ml amounts 12 hours before and 12, 36,

Table 1. Suppression by intravenous antigen and passive immunization of induction of delayed hypersensitivity. Sensitization was by intradermal injection of 0.5 ml of a mixture of equal parts of FCA and 5 percent SRBC in saline. The antigen was 1.0 ml of 5 percent SRBC in saline; it was given 24 hours before sensitization. Passive immunization was with hyperimmune-rat antiserum to SRBC, given intravenously in 1.0-ml quantities 12 hours before and 12, 36, and 60 hours after sensitization. Increases in paw volume are the means measured 21 hours after challenge on the 9th day after sensitization.

Rats (No.)	Sensitization	Antigen	Antiserum	Paw increase (%)
142	+	0	0	21
51	+	+	0	8
15	+	0	+	9
27	+	+	+	2
92	0	0	0	2

and 60 hours after sensitization (*6*). Challenge responses of these rats also were reduced in severity by about 60 percent. Development of hypersensitivity was abolished by combination of these two precedures of injection of antigen and passive immunization. These observations are summarized in Table 1; individual responses of suppressed and control rats are shown in Fig. 1.

Induction of hypersensitivity was not completely suppressed by other immunization procedures. For example, intravenous SRBC, given 2, 3, or 4 weeks before SRBC with FCA, partially suppressed sensitization. Intravenous SRBC, given both 3 weeks and 1 day before SRBC with FCA, produced still greater but incomplete suppression of sensitization. Serum obtained from rats recently injected with SRBC did not suppress sensitization. Large pools of serums were obtained from rats 1, 3, or 5 days after intravenous injection of 1.0 ml of 5 percent SRBC. Each rat received a total of 6 or 7 ml, from a serum pool, given in divided doses from 4 hours before to 60 hours after sensitization by SRBC with FCA; none of the serums measurably suppressed development of hypersensitivity.

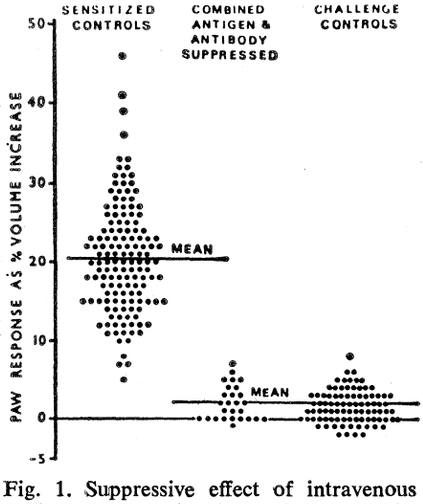

Fig. 1. Suppressive effect of intravenous antigen and passive immunization by induction of delayed hypersensitivity (individual responses of rats in selected groups are shown in Table 1). Each dot represents the response of a single rat as the percentage of increase in paw volume measured 21 hours after challenge on the 9th day after sensitization. The nonspecific responses produced by the challenge injection in normal animals (challenge controls) are equivalent to those in rats treated with antigen and antibody combined.

One could sustain suppression of hypersensitivity in rats injected repeatedly with SRBC plus FCA. Twelve rats were treated with a combination of intravenous antigen and passive immunization at the time of sensitization with SRBC plus FCA. All of six of them, injected weekly with SRBC plus FCA, had severe challenge responses to SRBC by 6 weeks; in contrast, none of the other six, injected intravenously with 1.0 ml of 5 percent SRBC 1 day before each weekly injection of SRBC with FCA, had detectable challenge responses 6 weeks later when the experiment was discontinued.

The procedures suppressing development of hypersensitivity were immunologically specific. For example, passive immunization with hyperimmune-rat antiserum to human erythrocytes or to *Bordetella pertussis* vaccine had no suppressive effect on sensitization to SRBC. Similarly, intravenous injection of human erythrocytes 1 day before sensitization with SRBC plus FCA did not suppress development of hypersensitivity to SRBC, although this procedure did suppress development of hypersensitivity to human erythrocytes in other rats injected with human erythrocytes plus FCA (*4*).

Either passive immunization or intravenous antigen partially suppressed induction of delayed-type hypersensitivity. Antibody interacting with antigen, or possibly with "antigen-reactive cells," may limit proliferation of cells mediating delayed-type hypersensitivity. Intravenous antigen may commit antigen-reactive cells to stimulate proliferation of cells producing antibody, and in this way reduce the number of antigen-reactive cells available to stimulate a hypersensitive response (*7*). The combined treatment presumably summates these two effects.

For maintenance of suppression of hypersensitivity, continuous or repeated antigenic stimulation is essential, but the role of antibody is difficult to assess. Animals with sustained suppression of hypersensitivity to SRBC have circulating antibody to SRBC, first from passive immunization and later from active production. Whether continued antibody interaction with antigen or responding cells is necessary for maintenance of suppression of the hypersensitive response is not known. Once hypersensitivity is induced, however, antibody has little or no suppressive effect; indeed, severe hypersensitivity is maintained by repeated injections of antigen with adjuvant when circulating-antibody titers to the antigen are very high.

The procedures we describe for preventing development of hypersensitivity are applicable to the problem of transplantation only when the antibody does not injure grafts. Antibody to antigens of an allograft may enhance growth of the graft (*8*), the indication being that antibody is not necessarily detrimental to survival of a graft. Indeed, survival of renal allografts has been prolonged by use of this general method of injection of antigen and passive immunization (*9*).

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6. Rats were sensitized with SRBC plus FCA and 5 weeks later were injected intravenously with SRBC alone. Pooled antiserum was obtained 8 days after intravenous antigen was given. The agglutinin or hemolysin titer of the serum for SRCB was 1 : 5120; treatment of the serum with 2-mercaptoethanol did not reduce the agglutinin titer.
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sensivity and antibody formation has been considered (*4*). "Antigen-reactive cell" is used in the sense proposed by J. F. A. P. Miller, *Lancet* **1967-II**, 1299 (1967). Selective suppression of delayed-type hypersensitive reactions by antigen or antibody alone has been described: J. R. Battisto and J. Miller, *Proc. Soc. Exp. Biol. Med.* **111**, 111 (1962); G. L. Asherson and S. H. Stone, *Immunology* **9**, 205 (1965); G. L. Asherson, *ibid.* **10**, 179 (1966); H. F. Dvorak, J. B. Billote, J. S. McCarthy, M. H. Flax, *J. Immunol.* **97**, 106 (1966); J. W. Streilein and E. A. Hildreth, *ibid.* **96**, 1027 (1966); A. J. Crowle and C. C. Hu, *Proc. Soc. Exp. Biol. Med.* **127**, 190 (1968).

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Behavioral Effects in Monkeys of Racemates of Two Biologically Active Marijuana Constituents

Abstract. *Both* dl-Δ^8- *and* dl-Δ^9-*tetrahydrocannabinol produced marked alterations of behavior in rhesus and squirrel monkeys. Squirrel monkeys appeared to have visual hallucinations. Continuous avoidance behavior of squirrel monkeys was stimulated by both drugs, but high doses of* dl-Δ^9-*tetrahydrocannabinol also caused depression after the stimulant phase. Complex behavior involving memory and visual discrimination in rhesus monkeys was markedly disrupted by both drugs.*

Pharmacologic studies of *Cannabis sativa* (marijuana, hashish) in animals have produced inconsistent results (*1–3*). Hashish induced aggressiveness (fighting) in one study (*2*) but prevented aggressive behavior in another (*4*). Some work has indicated that hashish produced analgesia, but this effect was not always found (*3, 4*). Apart from these diverse findings the main variable requiring control is the purity or composition of the hashish (*5, 6*). The actual amounts of the tetrahydrocannabinols (THC) contained in the hashish administered to animals have been difficult to determine because THC decomposes when exposed to air (*7*). A standard tetrahydrocannabinol preparation is needed to aid in the evaluation of the action of marijuana or hashish. Fahrenholtz, Lurie, and Kierstead (*8*) have synthesized crystalline *dl*-Δ^9-tetrahydrocannabinol, the racemate of the major active component of marijuana, and *dl*-Δ^8-tetrahydrocannabinol (an oil), which is the racemate of a minor active component of marijuana. Our results were obtained with these two racemates (*9*), and they indicate that both *dl*-Δ^8- and *dl*-Δ^9-tetrahydrocannabinols (hereafter termed Δ^8-THC and Δ^9-THC) are potent psychotropic agents producing pronounced behavioral aberrations.

Behavioral effects of these tetrahydrocannabinols were measured with operant conditioning techniques. The conditioning procedures used were: (i) continuous avoidance in squirrel monkeys (*10*), (ii) shock titration in squirrel monkeys (*11*), and (iii) delayed matching in rhesus monkeys (*12*). Several doses of both Δ^8- and Δ^9-THC were tested in from 4 to 11 subjects in each procedure. Generally, animals were drugged only once every 2 weeks, and no animal was ever drugged more frequently than once a week. For injection, Δ^9-THC was prepared by suspending it in 5 percent gum arabic alone or in gum arabic after initial mixing in three drops of glycerin or sesame oil; Δ^8-THC was suspended in gum arabic, but only after much levigation with glycerin or sesame oil. Before being prepared for injection the drugs were stored at −70°C, and care was taken that samples had minimum exposure to air (*13*).

The effects of Δ^9-THC in the continuous avoidance procedure are difficult to describe. Intraperitoneal doses of 4 or 8 mg/kg decreased the response rate to about 50 percent of the individual subject's own control rates. However, as the dose was further increased to 16, 32, and 64 mg/kg, the animals were frequently stimulated. They responded at about twice (200 percent) their control rates. This increased lever-pressing was not seen in all animals perhaps for reasons given below.

Observed changes in the general behavior of squirrel monkeys given Δ^9-THC are more relevant than changes in lever-pressing in this case. Monkeys given 4 or 8 mg/kg of Δ^9-THC sat quietly near the levers with head down and seemingly peered at the lower part of the box. Dosages of 16 mg/kg stimulated or excited the monkeys and caused them to walk about the box, apparently looking at something the experimenters did not see, or to crouch and move their heads from side to side and up and down as if watching some moving object. Some animals had a blank expression and gazed into space. We assumed that the animals had visual hallucinations, but the extent to which THC affects the oculomotor or other visual systems is unknown. In some monkeys when the dose of Δ^9-THC was 16 mg/kg, and in all monkeys given 32 or 64 mg/kg, this apparent hallucinatory reaction was more obvious. Monkeys moved quickly about the box, looked above and behind themselves, seemed to be in a state of panic (*14*), and appeared to fight with imaginary objects; their arms would swing rapidly through the air and they would attempt to grasp objects that were not there. These movements were rapid and associated with fine hand tremors. The fighting and swatting movements appeared well coordinated. However, it was impossible to determine whether some of these movements were completely voluntary. The animals tended to maintain one or two limbs in an unusual position; for example, one hind leg flexed against the abdomen. The subjects also tended to look intently at their widely opened hands; then one of the hands (sometimes both) was partly closed with palm up, and it was then held near the chest for several hours. The onset of the effect after small doses (4 to 8 mg/kg) of the drug was gradual, and about 1 hour was required after injection until behavior was clearly altered. Higher doses were active within 20 minutes. The stimulant phase of the drug action persisted about 3 hours and was followed by a period of depression (the animals assumed a crouched position and remained almost motionless). This depression lasted for 1 to 2 days and occasionally for a week. Nine subjects died after being severely depressed for 24 to 72 hours (*15*).

At intraperitoneal doses of 2, 4, and 8 mg/kg, Δ^8-THC increased the rate of lever-pressing in monkeys in the avoidance procedure. In contrast to Δ^9-THC, Δ^8-THC did not decrease lever-pressing at lower doses, and the stimulation produced by higher doses of Δ^8-THC was not followed by depression or death. The same type of bizarre effects were produced by both drugs, except that the effects of Δ^8-THC were somewhat more pronounced and typically were