

Further Studies of Streptococcal Infection in Normal and Splenectomized Monkeys* (32757)

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Previous studies from this laboratory have demonstrated similar responses in normal and splenectomized monkeys (1-3) and mice (4) following aerosol or intravenous challenge with streptococci, staphylococci, or pneumococci. In studies employing streptococci (1), intravenous challenge in monkeys was conducted 24-90 days after surgery. The present study was conducted to ascertain whether differences in susceptibility could be detected following a shorter postsplenectomy interval. Thus, normal and splenectomized monkeys were challenged intravenously 5, 8, or 12 days postsplenectomy with the same strain of streptococcus used previously. In addition, ancillary studies in normal monkeys with a second, more mouse-virulent streptococcal strain are described.

Materials and Methods. Young prepubertal monkeys (*Macaca mulatta*) were splenectomized¹ while under pentothal-ether anesthesia. In controls, the spleen was manipulated but not removed. Suspensions of *Streptococcus hemolyticus*, Group A, Strain S23 (5) and the Stollerman T14 strain were prepared and given intravenously as described previously (1). Base-line studies included physical examinations, hematologic, and serologic studies for 2 weeks prior to splenectomy and during the period between splenectomy and challenge. Monkeys were examined twice daily after challenge. Femoral vein blood was used for blood counts, cultures and C-reactive protein (CRP) tests daily or every other day and weekly for antistreptolysin O (ASO) titrations. Laboratory procedures used have been described previously (1). Complete autopsies were performed on all fatally-infected animals.

Exposure of normal monkeys to aerosols of the Stollerman T14 strain of *S. hemolyticus* was conducted in a model 3 Henderson apparatus (6).

Results. Four separate experiments (Table I) were conducted to compare the response of splenectomized and sham operated monkeys after intravenous challenge 5, 5, 8, and 12 days, respectively, postsplenectomy. In Exp. 1 (Table I) 4 splenectomized and 2 control monkeys each received 2.0×10^9 streptococci 5 days after surgery. All 6 appeared extremely lethargic, weak, and anorectic on the day after challenge, and all showed positive blood cultures and CRP tests, and polymorphonuclear leukocytosis. Two of the 4 splenectomized monkeys and both controls were febrile. All 4 splenectomized monkeys became progressively worse and died by the second post-challenge day. At autopsy, the streptococcus was isolated from heart blood, lung, liver, kidney, adrenals, brain, and bone marrow of all 4. One (no. 54) of 2 controls was lethargic, weak, and anorectic for 10 days, and on day 11 a 2-cm area of cellulitis, from which the streptococcus was isolated, was noted on the site of intravenous (saphenous) inoculation. The monkey became worse during the next 3 days and died on day 15. It was febrile on the first 2 postchallenge days only, but positive blood cultures, CRP tests, and leukocytosis ($21,000-42,000/\text{mm}^3$) were noted from the first day to death. Hemoglobin and hematocrit dropped from pre-challenge values of 11.7-12.4 gm and 37.0-41.5%, respectively, to 7.3 gm and 27.0%, respectively, just prior to death. Streptococci were isolated from most of the major organs at autopsy. The second sham operated monkey (no. 29) was lethargic, weak, and anorectic for 18 days, suddenly became worse on day 19, and died on day 21. Intermittent fever was observed for the first 9 days, and although blood cultures were positive for the first 11 days only, positive CRP tests, leukocytosis, and anemia were noted up to the time of death. On day 17, a 2.5×3.5 -cm area of cellulitis was observed at the site of

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TABLE I. Effect of Intravenous Streptococcal Challenge in Normal and Splenectomized Rhesus Monkeys.

	Spln.		Controls		Spln.		Controls		Totals (4 expts.)					
	Expt. 1. 5 days postsplen. (Dose: 2.0×10^9)				Expt. 2. 5 days postsplen. (Dose: 7.2×10^9)				Splen.	Controls				
Monkey no.	96	88	13	22	54	29	50	48	55	23	A4	C7		
Day of death	2	2	2	2	15	21	—	—	2	7	2	—		
Illness (days)	2	2	2	2	15	21	51	14	2	7	2	7		
Fever	—	+	—	+	+	+	—	+	+	—	—	+		
Leukocytosis	+	+	+	+	+	+	+	+	+	+	+	+		
CRP	+	+	+	+	+	+	+	+	+	+	+	+		
Blood culture	+	+	+	+	+	+	+	+	+	+	+	+		
ASO titer rise	ND ^a	ND	ND	ND	5 ^b	6	9	8	ND	ND	ND	5		
	Expt. 3. 8 days postsplen. (Dose: 6.4×10^9)				Expt. 4. 12 days postsplen. (Dose: 2.3×10^9)									
Monkey no.	46	5-9	A5	A54	51	A46	A11	43	A7	89	A22	94		
Day of death	2	2	2	—	2	—	1	2	—	—	2	—	11/16 ^c	5/8
Illness (days)	2	2	2	13	2	42	1	2	14	18	2	6	16/16	8/8
Fever	+	—	+	+	—	+	—	+	+	+	+	+	10/16	6/8
Leukocytosis	+	+	+	+	+	+	+	+	+	+	+	+	16/16	8/8
CRP	+	+	+	+	+	+	+	+	+	+	+	+	16/16	8/8
Blood culture	+	+	+	+	+	+	+	+	+	+	+	+	16/16	8/8
ASO titer rise	ND	ND	ND	6	ND	6	ND	ND	7	8	ND	11	5/5	5/5

^a ND = not done.

^b Tubes increase in titer.

^c Numerator = no. positive; denominator = total no.

intravenous inoculation from which the streptococcus was isolated on days 17 and 18. At autopsy, however, none of the major organs yielded streptococci.

Thus, in Expt. 1, all 4 splenectomized monkeys were dead by the second postchallenge day, whereas 2 sham operated controls survived for 15 and 21 days, respectively. These results suggested repetition of the experiment.

Accordingly, in Expt. 2 (Table I), 4 monkeys splenectomized 5 days previously and 2 controls were inoculated intravenously with 7.2×10^9 streptococci. One (no. 55) of 4 splenectomized monkeys was acutely ill, febrile, and exhibited positive blood cultures and CRP tests, and leukocytosis on the day after challenge, and died on the second day. A second splenectomized monkey (no. 23) showed no symptoms on the first postchallenge day, was lethargic, weak, and anorectic on the second day, became worse on the third day and died on day 7. Although rectal temperatures were normal during the 7-day pe-

riod, positive blood cultures and CRP tests, leukocytosis, and anemia were observed from the first day to death. Group A streptococci were isolated from most of the major organs of both monkeys at autopsy. One (no. 48) of the remaining 2 splenectomized animals was extremely lethargic, weak, and anorectic during the first 4 days postchallenge, began to improve slowly on the 5th day and was normal by day 15. Fever was noted on the first day only. Blood cultures and CRP tests were positive for 15 and 17 days, respectively, and leukocytosis was observed for 11 days, but anemia persisted for about 31 days. The fourth splenectomized monkey (no. 50) was lethargic, weak, and anorectic, although afebrile, for about 6 weeks after challenge, and did not appear well until about day 52. Three areas of cellulitis each measuring 2.0-5.0 cm in diameter were observed on the left leg at the site of intravenous inoculation, on the right knee, and on the right elbow on days 7 through 21, 25 through 32, and 31 through

52 days, respectively; streptococci were isolated from all 3 lesions. Blood cultures were positive for 31 days, but CRP tests were negative after day 11. Leukocytosis and anemia were observed continuously from days 1 through 42. The ASO titers in the 2 surviving splenectomized monkeys rose from 1:10 and 1:40, respectively, before challenge to 1:5120 and 1:10,240, respectively, 2 weeks after challenge.

One (no. A4) of 2 sham operated monkeys was acutely ill, although afebrile, and showing positive blood cultures and CRP tests, leukocytosis, and anemia on the day after challenge; it died on the second day. The other (no. C7) was lethargic, weak, anorectic, and febrile for 3 days, began to improve rapidly on the fourth day, and was normal by the eighth day. Positive blood cultures and CRP tests, leukocytosis, and anemia were observed for 17, 11, 11, and 28 days, respectively. The ASO titer increased from 1:40 before challenge to 1:1280 2 weeks after challenge.

Thus, in Expt. 2, 2 of 4 monkeys splenectomized 5 days previously died 2 and 7 days, respectively, postchallenge and 1 of 2 sham operated animals died on the second day. The 1 surviving control was fully recovered by the eighth day, whereas, the 2 splenectomized monkeys that survived were not normal until days 15 and 52, respectively.

In Expt. 3 (Table I), 4 splenectomized and 2 sham operated monkeys each received 6.4×10^9 streptococci intravenously 8 days after surgery. All 6 were acutely ill on the day after challenge and 3 of 4 splenectomized and 1 of 2 controls were dead by the second day. Clinical, laboratory, and autopsy findings were similar to those in rapidly fatal infections in previous experiments. The fourth splenectomized monkey (no. A54) was febrile for the first 5 days after challenge, but began to show clinical improvement on the second day and was normal by day 14. However, blood cultures were positive continuously from days 1 through 35. Positive CRP tests, leukocytosis, and anemia were observed for 14, 22, and 14 days, respectively. Thus, this animal showed positive blood cultures for about 3 weeks after it appeared

normal and for about 2 weeks after other laboratory tests were within normal limits. The ASO titers rose from 1:10 prior to challenge to a peak of 1:640 at 5 weeks. The surviving sham operated monkey no. (A46) was lethargic, weak and anorectic for 26 days after challenge, began to improve slowly, and was not fully recovered until day 43. Three areas of cellulitis yielding streptococci and measuring 2.5–6.0 cm in diameter were observed on the left ankle, right leg at the site of intravenous inoculation, and on the right hip, respectively, 26–37, 14–35 and 14–35 days, respectively, after challenge. Fever was noted for the first 2 postchallenge days only. Blood cultures and CRP tests were positive for 35 days, and leukocytosis and anemia were observed for 28 and 22 day, respectively. The ASO titer increased from 1:10 prior to challenge to 1:640 at 3 weeks.

In Expt. 4 (Table I), 4 monkeys splenectomized 12 days previously and 2 controls each were inoculated intravenously with 2.3×10^9 organisms. Two of 4 splenectomized and 1 of 2 controls were acutely ill on the day after challenge and all 3 were dead by the second day. One (no. A7) of the remaining 2 splenectomized monkeys was only mildly ill on the first day but was very lethargic, weak, and anorectic on the second day. Recovery began on day 8 and was complete by day 15. It was febrile on the first 2 days only, and positive blood cultures and CRP tests, leukocytosis, and anemia were observed for 14, 10, 10, and 14 days, respectively. The other splenectomized monkey (no. 89) was extremely lethargic, weak, and anorectic on the day after challenge and remained so through day 5. Subsequent recovery was slow and not complete until day 19. Intermittent fever was observed during the first 15 days. Although all tests for CRP were negative after day 11, blood cultures were positive through day 31. Leukocytosis and anemia were noted for 22 days. The ASO titers in the 2 surviving splenectomized monkeys rose from 1:20 and 1:40, respectively, before challenge to 1:2560 and 1:10,240, respectively, at 2 weeks. The remaining sham operated monkey (no. 94) showed no symptoms on the day after challenge except

TABLE II. Comparison of Response of Normal Monkeys after Intravenous Challenge with S23 and T14 Strains of *S. hemolyticus*, Group A.

Group:	A				B				C			
	T14		S23		T14		S23		T14		S23	
Challenge dose:	1.3×10^9		1.1×10^9		1.3×10^8		1.1×10^8		1.3×10^7		1.1×10^7	
Monkey no.	25	27	91	68	85	12	15	19	28	A27	24	26
Day of death	1	2	2	2	—	3	—	—	—	—	—	—
Illness (days)	1	2	2	2	14	3	2	2	—	3	—	—
Fever	—	—	—	—	+	—	+	—	+	+	—	—
CRP	+	+	+	+	+	+	+	+	+	+	—	—
Blood culture	+	+	+	+	+	+	+	—	—	+	—	—
ASO titer rise	ND ^a	ND	ND	ND	5 ^b	ND	5	5	2	6	3	7

^a ND = not done.

^b Tubes increase in titer.

for fever which lasted 4 days. It was only mildly lethargic and weak on day 2 through 6, and appeared normal on day 7 and thereafter except for enlargement of inguinal nodes from days 12 through 17. However, blood cultures and CRP tests were positive for 42 and 24 days, respectively. Leukocyte counts were elevated for 24 days and anemia persisted for 49 days. The ASO titer rose from 1:10 to a peak of 1:20,480 at 2 weeks.

Ancillary studies in normal monkeys with S. hemolyticus, Strain T14; Intravenous challenge. Preliminary studies showed that as few as 10–20 streptococci of the T14 strain were lethal for white mice inoculated intraperitoneally, whereas 200–400 were required with the S23 strain (1). Virulence for monkeys was compared by intravenous inoculation of graded doses of each strain, as shown in Table II. Each of 2 pairs of monkeys (Group A) receiving 1.3×10^9 T14 cells and 1.1×10^9 S23 cells, respectively, were acutely ill on the day after challenge and all 4 were dead by the second day. Clinical, laboratory, and autopsy findings were similar in the 2 pairs, and similar to those in previous experiments. In Group B, Table II, 1 (no. 12) of 2 monkeys given 1.3×10^8 T14 cells died on the third day, whereas the other (no. 85) was febrile, lethargic, weak, and anorectic for 14 days and exhibited positive blood cultures and CRP tests for 21 and 28 days, respectively. In contrast, neither of 2 monkeys receiving 1.1×10^8 S23 cells showed more than mild lethargy on the first 2 days

after challenge and 1 of 2 was febrile on the second day only. One (no. 15) of 2 had a positive blood culture on the third day only, whereas all cultures were negative in the other (no. 19). The CRP was positive on the third day only in both monkeys. In Group C, Table II, 1 (no. A27) of 2 monkeys given 1.3×10^7 T14 cells was febrile, lethargic, and mildly anorectic for only 3 days, but showed positive blood cultures and CRP tests for 10 and 21 days, respectively. The other (no. 28) showed no symptoms after challenge, was febrile on the second day only, and all blood cultures were negative. A CRP test was positive on the third day, however. Neither of 2 monkeys receiving 1.1×10^7 S23 cells showed any symptoms, and all blood cultures and CRP tests were negative. All 7 surviving monkeys exhibited similar increases in ASO titer after challenge. These results suggested that in monkeys, as in white mice, the T14 strain was more virulent than the S23 strain.

Aerosol challenge. Eight monkeys exposed to an aerosol of the T14 strain each retained about 7.4×10^6 cells during a 3-min exposure in a model 3 Henderson apparatus. None became ill after challenge and all blood cultures and CRP tests were negative. However, 7 of 8 exhibited a significant, 4-fold or greater increase in ASO titer 2–3 weeks after exposure. Thus, the response was similar to that observed previously (1) in monkeys challenged by aerosol with the S23 strain.

Intravenous inoculation after previous

aerosol challenge. The above 8 monkeys that were exposed to an aerosol of the T14 strain were rechallenged, intravenously, 4 weeks later with 2.2×10^9 cells of the same strain. Seven control monkeys not exposed to the streptococcal aerosol were similarly challenged intravenously. All 15 were moderately to acutely ill on the day after challenge; the 2 groups were similar in this respect. All 7 controls were dead by the third postchallenge day, whereas only 3 of 8 previously exposed to the streptococcal aerosol were dead by the third day. Of the remaining 5, 1 and 2 monkeys died on days 9 and 13, respectively, and the other 2 survived. The 3 that died 9 or 13 days postchallenge showed positive blood cultures and CRP tests, leukocytosis, and anemia up to the time of death. One of 2 survivors exhibited fever continuously for the first 8 days and was extremely weak, lethargic, and anorectic for the first 6 days. Slow recovery beginning on day 7 was not complete until day 18. However, all blood cultures were negative after the second day and positive CRP tests, leukocytosis, and anemia were noted only for 9, 7, and 11 days, respectively. The other survivor was lethargic, weak, and anorectic during the first 2 days, then began to recover rapidly, and was normal by day 12. Fever was noted for 5 days and blood cultures and CRP tests were positive for 9 and 13 days, respectively. Leukocytosis and anemia persisted for 13 and 21 days, respectively.

These results suggested that antecedent exposure to the streptococcal aerosol resulted in some modification of the response to subsequent intravenous challenge with the homologous strain. There was no correlation, however, between ASO titer after aerosol exposure and response after intravenous rechallenge. Titers of 1:160, 1:640, and 1:1280 were observed at the time of intravenous rechallenge in 3 monkeys that were dead by the third day. Three that died on days 9, 13 and 13 showed prechallenge titers of 1:640, 1:80, and 1:320, respectively, and prechallenge titers of 1:640 and 1:2560 were noted in the 2 survivors, respectively.

Discussion. These studies demonstrated that normal monkeys challenged, intravenously, with the T14 strain of a Group A hemolytic streptococcus showed a similar infection pat-

tern to that observed previously (1) with the S23 strain of a Group A streptococcus. Comparison suggested that the T14 strain was somewhat more virulent. However, following aerosol challenge, no obvious clinical evidence of infection was noted as with previous studies with the S23 strain, although significant ASO titer increases were observed in 7 of 8. This aerosol exposure was followed by a modified host response when intravenous challenge was instituted 4 weeks later. Whereas all 7 controls, not previously exposed to an aerosol died by the third day, only 3 of 8 "experienced" monkeys were dead at this time; fatal infection did not occur in 3 other monkeys until days 9 to 13 and the remaining 2 recovered spontaneously.

Previous studies (1) comparing the response of splenectomized and sham operated monkeys following intravenous challenge with the S23 strain of streptococcus 24-90 days after surgery revealed no differences in response. In the present study with the same streptococcus again no differences were observed in monkeys challenged 8-12 days after surgery. However, following challenge 5 days after surgery, some differences were observed. Although death of 6 of 8 splenectomized and 3 of 4 control monkeys demonstrated similar eventual mortality results, in one experiment all 4 splenectomized monkeys died on the second day while the 2 controls died on days 15 and 21, respectively. In a second similar experiment the 2 of 4 splenectomized monkeys which survived were ill for 14 and 51 days, respectively, while 1 of 2 controls which survived was ill for only 7 days. In previous studies showing the comparable response following pneumococcal infections in normal and splenectomized monkeys (2) the time interval following surgery was 4 days in one experiment. At this period no differences were observed following inoculation of 6.2×10^9 type III pneumococci. No mortalities occurred in the 4 splenectomized and 2 control monkeys, and duration of clinical illness was 2-14 and 9-11 days, respectively. Blood cultures in the 4 splenectomized monkeys were positive for 7, 10, 14, and 35 days, respectively, and 21 and 56 days, respectively, in the 2 controls. Thus, the cumulative evidence of our studies in monkeys with streptococci, pneumococci,

and staphylococci would suggest that the end result in reference to mortality is the same in splenectomized and sham operated animals at periods varying from 4 to 255 days post-splenectomy. Whether the more fulminating illness observed at 5 days but not 8–12 days, postsplenectomy, following streptococcal infection was related to the specific organism, the absence of the spleen per se, or the more recent time interval since major surgery, i.e., removal of the spleen as compared to sham operation is not clear and bears further investigation.

Conclusions. Intravenous challenge of rhesus monkeys with T14 and S23 strains of Group A hemolytic streptococci was followed by similar clinical and laboratory evidence of infection, and the former strain appeared somewhat more virulent. Aerosol challenge with the T14 strain produced no demonstrable disease but did offer some partial protection to subsequent intravenous challenge 4 weeks

later. No significant differences were noted between splenectomized and normal monkeys in reference to fatal outcome after challenge 5–12 days after surgery. However, splenectomized monkeys challenged at the 5-day period either died earlier, or if they survived had longer periods of clinical illness, than their nonsplenectomized controls.

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Effect of Pretreatment with Androgen in the Neonatal Rat on LH-Induced Ovarian Cholesterol Depletion* (32758)

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The ovarian cholesterol depletion (OCD) assay for LH reported by Bell *et al.* (1) has not been usable in this and other laboratories (2) because of the multiphasic nature of the dose-response curve. We have demonstrated that ovarian cholesterol levels in PMS-HCG treated Wistar rats exhibit a diurnal rhythm, and that this rhythm may reflect endogenous LH release (3). This could then account for the multiphasic nature of the curve obtained in the assay. We have also shown that androgen sterilization will block this diurnal rhythm (3). The present study was designed to determine the effect of LH on ovarian cholesterol levels in the androgenized rat pretreated with PMS and HCG.

Method and Materials. All rats used in this study were of the Purdue-Wistar strain. The

rats were housed six per cage, and kept in a controlled environment of 72–74°F. with a relative humidity of 45–55%. The light and dark cycle consisted of 13 hours of light and 11 hours of darkness with the light cycle starting at 7:00 a.m. Food and water were given *ad libitum*.

The PMS-HCG¹ treatment consisted of subcutaneous injections of 50 IU of PMS on days 22 and 24 of age followed by 25 IU of HCG on day 26. Both hormones were dissolved in physiological saline and injected in a 0.1 ml volume. Pretreatment with androgen consisted of a single subcutaneous injection of 1.25 mg of testosterone propionate on day 2 of age.

Ten days following PMS-HCG treatment

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