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ETIOLOGY OF OROYA FEVER

XV. Effect of Immune Serum on the Course of Bartonella bacilliformis Infection in Macacus rhesus

By HIDEYO NOGUCHI, M.D., HENRY R. MULLER, M.D., EVELYN B. TILDEN, Ph.D., and JOSEPH R. TYLER

(From the Laboratories of The Rockefeller Institute for Medical Research)

Plates 19 and 20

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Very early in the course of the work on Carrion's disease, experiments were begun (Noguchi) to test the effect of an immune serum, prepared in rabbits, or obtained from recovered monkeys, on the course of experimental infection with *Bartonella bacilliformis* in *Macacus rhesus*. While these early results were not altogether promising, owing probably to the use of small doses of immune serum, there was some indication that the introduction of the serum simultaneously with the infective material inhibited the development of the infection, at least, temporarily (Table 1), the controls showing skin lesions and positive blood culture earlier than the treated animals.

Recently, studies were undertaken to determine the effects of larger doses (20 cc.) of convalescent serum given 24 hours before intradermal and intravenous inoculation of a highly virulent strain of *Bartonella bacilliformis* isolated from phlebotomi.[1] This procedure was found to have a marked inhibitory effect (Table 2). While the control animal developed severe skin lesions within 2 weeks, and its blood yielded cultures of *Bartonella bacilliformis* in a dilution of 1:10,000 after periods of 10 and 17 days, the treated animals remained free from lesions for 25 days, and cultures of the blood were sterile. However, in two of the three treated animals, typical nodules eventually developed (after 25 days) at one or more sites of intradermal inoculation, and after 26 days the blood of one animal yielded cultures in a titre of 1:100. The third treated animal escaped infection.

[1] Noguchi, H., Shannon, R. C., Tilden, E. B., and Tyler, J. R., *J. Exper. Med.*, 1929, **49**, 993.

TABLE 1

M. rhesus No.	Date of inoculation 1926	Material inoculated	Mode of inoculation	Result		Treatment		Result	
				Local lesions	Blood culture	Date	Material	Local lesions	Blood culture
59	Oct. 14	0.5 cc. susp. nod. Rh. 54 + 1.5 cc. immune rabbit serum. 2 cc. serum intrav.	Intradermal. Scarification.	No reaction until 20 days	− 5 days + 1:100,000 20 days	Nov. 26	3 cc. immune rabbit serum	Regression began Dec. 2	− Nov. 26 − Dec. 4
60	" "	Same except only 1 cc. serum intrav.	Same	Sl. transient reaction at one site after 20 days	− 5 days + 1:1,000 20 days				
61	" "	0.5 cc. susp. nod. Rh. 54 + 1.5 cc. convalescent serum Rh. 18 2 cc. serum intrav.	Same	No reaction	− 5 days Died of secondary infection Oct. 31.				
3-T	" "	Same as 61, but 1.5 cc. serum intrav.	Same	All inoculations positive in 12 days, mature nodules 20 days	+ 1:100,000 5 days + 1:100,000 20 days				

57 Control	Oct. 14	Mixture 0.5 cc. nodule susp. Rh. 54 and 1.5 cc. *normal rabbit serum*	Same	Definite 9 days	+ 1:100 5 days − 43 days	Nov. 26 1.5 cc. immune rabbit serum and 1 cc. culture subc.	No change Nodules still present Dec. 13	− Dec. 4
2-T Control	" "	Same	Same	Definite 5 days	+ 1:100,000 5 days	See Etiology of Oroya Fever. XIII.		
1-T Control	" "	Nodule susp. Rh. 54	Same	Definite 8 days Well devel. 22 days	+ 1:1,000 5 days − 43 days	See Etiology of Oroya Fever. XIII.		
55 Control	" "	Same	Same	Definite 8 days. Well developed 13 days	− 5 days − 43 days			
83	*1927* Feb. 8	Susp. nod. tissue *M. rhesus* 78 Cultures P-5 strain 5 cc. immune serum intrav.	Intradermal, eyebrows and abdomen	No reaction	− 9 days − 23 "	Reinoculated Mar. 16, 1927, with suspension nodular tissue of *M. rhesus* 3A. No reaction.		
84A	" "	Same	Same	" "	− 9 days − 23 "	Killed because of tuberculosis Mar. 12, 1927.		
82	" "	Control, no serum	Same	" "	− 9 days − 23 days	" " " " " " "		
76	" "	Control, no serum	Same	Definite nodules 21 days. Reached 0.5 cm. in diameter	− 9 days + 1:100 23 days			

TABLE 2

Effect of One Intravenous Injection of Convalescent Serum 24 hrs. Prior to Inoculation

M. rhesus No	Pooled convalescent serum cc.	Date of inoculation 1928	Material inoculated	Mode of inoculation	Result	
					Local lesions	Blood culture
I-20	20	Nov. 10	Suspension nodular tissue from *M. rhesus* I-9 (Phleb. Str. 1)	Intradermal, 4 sites on abdomen. Scarification, 1 site Intrav. 1 cc.	One intradermal inoculation definitely positive after 25 days. Scarified area showed sl. reaction. Other 3 sites neg.	− 10 days − 17 " − 26 "
I-21	20	" "	Same	Same	All inoculations negative	− 10 days − 17 " + 1:100 26 days − 33 days
I-30	20	" "	Same	Same	2 intradermal positive after 25 days, 8 mm. in diameter.	− 10 days − 17 " − 26 " − 33 "
I-31	Control, no serum	" "	Same	Same	All intradermal inoculations positive in 14 days, reaching full development in 25 days. Severe reaction on scarified area. Died Déc. 10 (30 days)	+ 1:10,000 10 days + 1:10,000 17 "

Apparently the microorganisms had, in the two instances, remained dormant at the sites where they were intradermally introduced until the lapse of the period of passive immunity. This conclusion has been confirmed, by another experiment, as likewise the view that blood invasion was inhibited.

Four animals received an injection 24 hours before inoculation of 20 cc. of convalescent serum, another injection of the same amount 11 days after inoculation, and two subsequent injections of 4 cc. and 5 cc., respectively, 15 days and 26 days after inoculation. The control animals developed definite nodules in 11 days, which progressed rapidly and had reached very large size 12 days later. At this time the treated animals were still free from any lesions, and the blood was sterile. These conditions were maintained for a period of 38 days after inoculation (13 days after the last serum injection). At the end of this period, although blood cultures were still negative, definite nodules appeared at the sites of intradermal inoculation in 3 of the 4 monkeys; in the fourth animal nodules appeared 5 days later. The development of the lesions thereafter followed the usual course of experimental verruga, but *Bartonella bacilliformis* was recovered from the blood of the treated animals only in one animal (S-4) and then only once, 61 days after inoculation.

The experiment is recorded in detail in Table 3. It will be noted that the material used for inoculation was varied. Each monkey was injected intradermally at 3 or 4 sites on one side of the abdomen with a suspension of nodular tissue from a monkey infected with Phlebotomus Strain 1 of *Bartonella bacilliformis*, and each received similar inoculations on the other side with cultures of Phlebotomus Strain 3 or Strain 4. While the control animal for the nodule suspension responded in the usual way, only one of the 3 treated animals reacted to this material, though all showed the delayed reaction to the culture inoculations. But for the precaution taken to use varied material, the cultures in this instance proving to be of the maximum virulence, the results might have been interpreted as indicating that 3 of the 4 serum-treated animals were completely protected against infection. The one monkey which showed a delayed reaction (58 days) to the inoculation of the moderately virulent nodule suspension was evidently the most susceptible animal of the four, since it was the only one which was not completely protected against invasion of the blood by *Bartonella bacilliformis*.

The results recorded in Tables 2 and 3 indicate that while the

TABLE 3

Effect of Treatment with Convalescent Serum (First Dose Given 24 Hours Before Inoculation)

M. rhesus No.	Pooled convalescent serum cc. total	No. injections	Material and mode of inoculation	Local lesions	Blood culture
S-1	49	4	*Nodule susp. rhesus I-30* 3 sites intradermally	–	
			1 by scarification	–	– at 11, 19, 26, 46, 61 days after inoculation
			Cultures Phleb. Str. 1 3 sites intradermally	Very slight induration 39 days Mature in 66 " Recovery complete in 83 "	
			1 by scarification		
S-2	Same	Same	*Nodule susp. rhesus I-30* 3 sites intradermally	–	
			1 by scarification	–	– at 11, 19, 26, 46, 61 days after inoculation
			Cultures Phleb. Str. 3 3 sites intradermally	Definite nodules 39 days Mature in 51 " Recovery complete in 83 days	
			1 by scarification	+	
S-3	Same	Same	*Nodule susp. rhesus I-30* 3 sites intradermally	–	
			1 by scarification	–	– at 11, 19, 26, 46, 61 days after inoculation
			Cultures Phleb. Str. 4 3 sites intradermally	Very slight induration 39 days Mature in 66 " Recovery complete in 83 "	
			1 by scarification	–	

S-4	Same	Same	*Nodule susp. rhesus I-30* 3 sites intradermally 1 by scarification	Nodule at 1 site 58 days Mature in 98 " 2.5 cm., pedunculated Still 8 mm. at 111 days –	– at 11, 19, 26, and 46 days, + at 61 days after inoculation
			Cultures Phleb. Str. 4 3 sites intradermally 1 by scarification	Nodules at 2 of 3 sites in 46 days Mature in 83 " Recovery complete in 102 days –	
S-5 Control	None		*Nodule susp. rhesus I-30* 4 sites intradermally 1 by scarification	Definite nodules in 11 days Mature in 23 " Recovery complete in 58 days ++	+ at 11, 19, and 26 days, – at 46 days after inoculation
S-6 Control	None		*Cultures Phleb. Str. 3* 4 sites intradermally 1 by scarification	Definite nodules in 11 days Mature in 23 " Recovery complete in 65 days ++++	+ at 11, 19, and 26 days, – at 46 days after inoculation
S-7 Control	None		*Cultures Phleb. Str. 4* 4 sites intradermally 1 by scarification	Definite nodules in 11 days Mature in 23 " Recovery complete in 58 days ++	– at 11 days + at 19 and 26 days – at 46 days after inoculation

TABLE 4

Effect of Treatment with Convalescent Serum (First Dose Given 5 Days after Inoculation)

M. rhesus No.	Material and Mode of inoculation	Date 1929	Local lesions	Blood culture	Amounts of convalescent serum administered
S-8	Culture, Phlebotomus Strain 4.	Jan. 23	First definite nodule (4 mm.) 5 days after inoc.	+ 5 days	20 cc., 5 days after inoc.
			Nodules 10 mm., red, prominent, scarification +, 13 " " "	− 13 "	20 cc., 13 " " "
				− 30 "	10 cc., 16 " " "
			Nodules average 15 mm., scarification + 19 " " "	− 43 "	
			Nodules mature (10–20 mm.) 36 " " "		
			Regression complete 67 " " "		
S-9	Same		First definite nodule (4 mm.) 5 " " "	Same	Same
			Nodules 8–9 mm. 13 " " "		
			Same, scarification + 19 " " "		
			Nodules mature (13–15 mm.) 36 " " "		
			Regression complete 67 " " "		

convalescent serum was not potent enough to protect completely against the inoculation of highly virulent cultures, it had a marked inhibitory action, as shown by the long delay in the development of the lesions, and that only occasionally did it fail to prevent invasion of the blood by the Bartonella.

The effect of large doses of convalescent *rhesus* serum given after local lesions had started to develop following the inoculation of cultures, was tested as follows:

In two *rhesus* monkeys, S-8 and S-9, treatment with convalescent serum was started 5 days after intradermal inoculation with a virulent strain of *Bartonella bacilliformis*, that is, when definite nodules had appeared, and when blood culture in both animals was positive in a dilution of 1:10. The serum was given intravenously in a dose of 20 cc. Notwithstanding the treatment, the nodules progressed rapidly, but 8 days later blood cultures were negative. At this time a second intravenous injection of 20 cc. of convalescent serum was given to each animal. Blood cultures continued negative throughout the remaining course of disease, but the local lesions progressed, and each animal was given another intravenous injection of 10 cc. of the immune serum on the 17th day. There was no perceptible effect of the serum treatment on the growth of the nodules, which matured about the 36th day after inoculation. Recovery was complete on the 67th day. At no test after serum was given were positive blood cultures obtained.

This experiment, recorded in detail in Table 4, shows that convalescent serum, given after the development of the nodules, sterilized the blood stream but had no effect on the nodule formation.

SUMMARY

Experiments are reported on the effect upon the course of experimental verruga peruana in *Macacus rhesus* of the injection of (1) small quantities of rabbit immune serum simultaneously with living cultures, (2) one large dose of convalescent monkey serum 24 hours prior to inoculation, (3) a similar preliminary dose followed by three subsequent injections of the serum, (4) three large doses of convalescent serum, following the inoculation. The convalescent serum was found (1) to prevent the multiplication of *Bartonella bacilliformis* in the blood in most instances, and (2) to delay the development of the skin lesions for considerable periods, when given before inoculation. When the serum treatment was not begun

until after the appearance of the skin lesions, it had no effect on the progress of the nodules, although the blood became free from *Bartonella bacilliformis*.

Since the severe effects of verruga peruana (Carrion's disease) are believed to be due to the multiplication of *Bartonella bacilliformis* within the blood, the injection of convalescent serum in cases of Carrion's disease in man would appear to offer promise.

EXPLANATION OF PLATES

Plate 19

Figs. 1 and 2. Two of the control animals, S-7 and S-5, 24 days after inoculation.

Figs. 3 and 4. Treated animals, S-1 and S-2, 24 days after inoculation. Compare with Figs. 1 and 2.

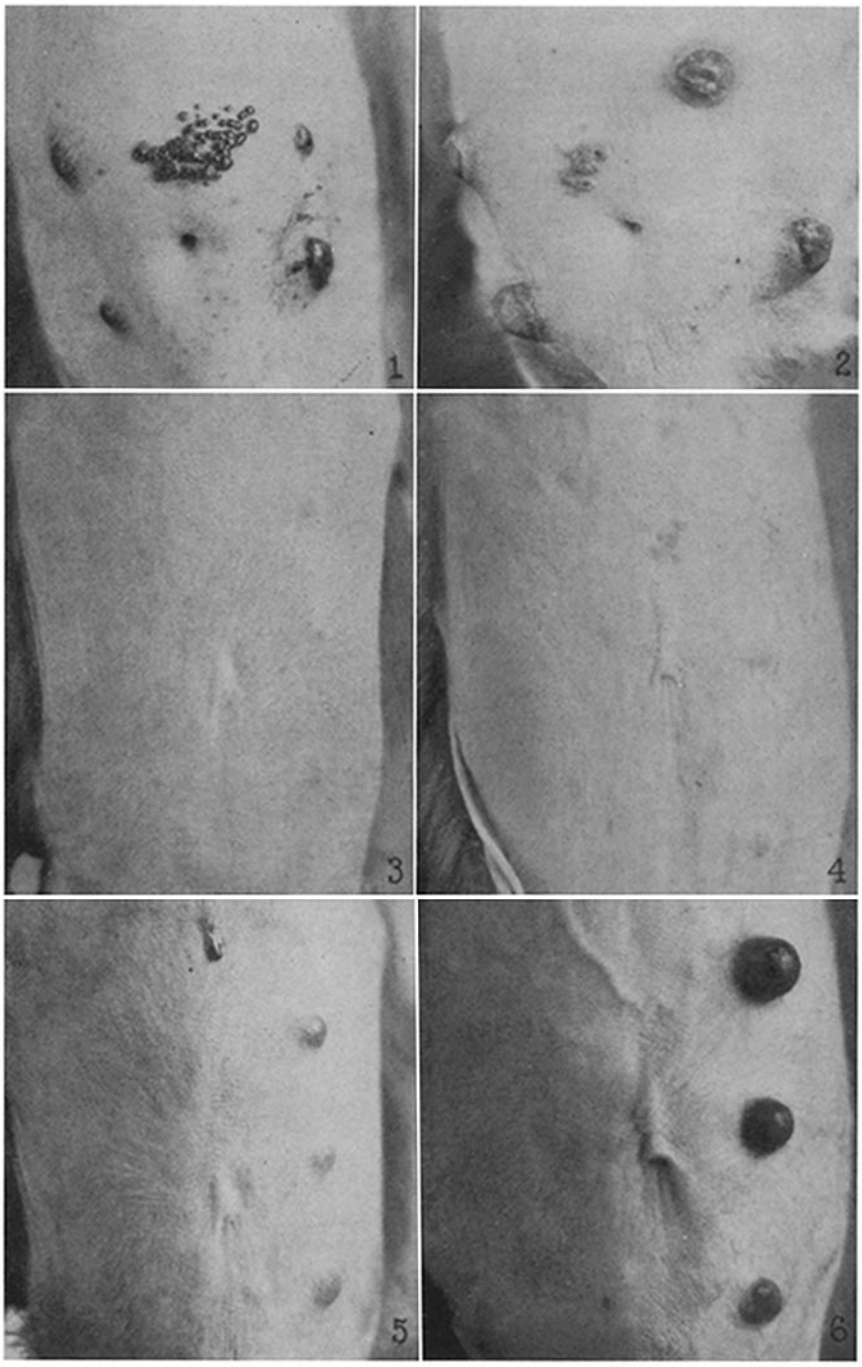
Figs. 5 and 6. Treated animals, S-1 and S-2, 54 days after inoculation, when the lesions were comparable with those of the controls at 24 days (Figs. 1 and 2).

Plate 20

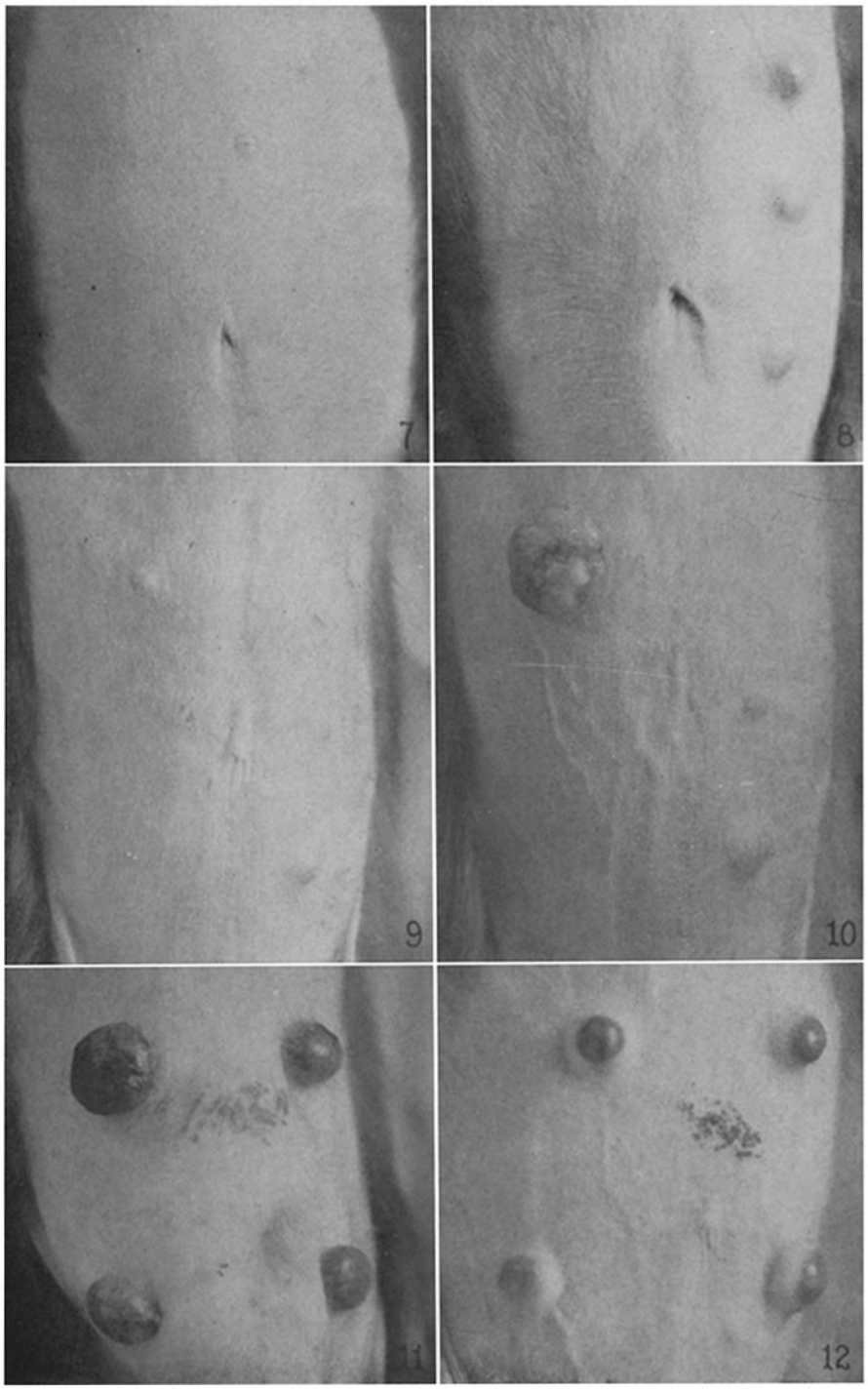
Figs. 7 and 8. Treated animal, S-5, 24 and 54 days after inoculation, respectively.

Figs. 9 and 10. Treated animal S-4, 66 days and 100 days after inoculation, respectively. At 66 days the lesions had just become definite.

Figs. 11 and 12. Rhesus S-8 and S-9 (treatment begun after development of the lesions), 22 days after treatment was begun.



(Noguchi *et al.*: Etiology of Oroya fever. XV)



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