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Local Shwartzman Phenomenon in Athymic Nude Mice (40827)

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The local Shwartzman reaction is classically produced by the intracutaneous injection of bacterial endotoxin, followed 24 hr later by the intravenous injection of bacterial endotoxin. The intracutaneous injection is referred to as preparatory, whereas the intravenous injection is referred to as provocative. Within a few hours after the provocative injection, an hemorrhagic necrotic lesion develops at the site of the initial intracutaneous injection of endotoxin. This reaction, which is actually generated by intravascular coagulation and occlusion of the capillaries and venules of the prepared skin site (1), is generally designated as the Shwartzman phenomenon. The Shwartzman reaction has most convincingly been demonstrated in rabbits, where it cannot only be provoked by an intravenous injection of endotoxin, but also by dsRNAs (double-stranded RNAs) such as $(I)_n \cdot (C)_n$ (polyinosinic acid·polycytidylic acid) (2–4). Macroscopically, the local Shwartzman reaction is often recognized as edema, erythema, and/or induration (3, 4). Herein we demonstrate that athymic nude [(nu/nu) (5)] mice are particularly prone to the Shwartzman reaction, and that in these mice, as in rabbits, the reaction can be provoked by both endotoxin and dsRNA.

Materials and methods. The nude (nu/nu) mouse strain used in our studies was of a predominantly NMRI background as has been documented before (6). The mice were bred according to breeding scheme IV of Giovanella and Stehlin (7). They were reared and maintained in conventional conditions, and submitted to the tests when 25–30 days old (weighing 16–18 g). The origin and abbreviations of the test compounds were as follows: *Salmonella enteritidis* endotoxin (ESE): Difco Laboratories, Detroit, Michigan; bovine serum albumin (BSA): Serva Feinbiochemica, Heidelberg, West Germany; Dulbecco's phosphate-buffered saline, containing both Mg^{2+} and Ca^{2+} (PBS); polyinosinic acid·polycytidylic acid $[(I)_n \cdot (C)_n]$: P-L Biochemicals, Milwaukee, Wisconsin; polyinosinic acid·poly(2-thiocytidylic acid) $[(I)_n \cdot (s^2C)_n]$ (8): kindly provided by K. Reuss (E. Merck, Darmstadt, W. Germany); f_2 dsRNA, extracted from *Escherichia coli* infected with f_2 phage (9): kindly provided by L. Borecky (Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslovakia); PC dsRNA, extracted from the mycelial macerate of the mold *Penicillium chrysogenum* (10): generously supplied by R. J. Douthart (Lilly Research Laboratories, Indianapolis, Indiana); Newcastle disease virus (NDV), Kumarov strain: propagated in the allantoic fluid of 10-day-old embryonated eggs; *Brucella abortus* (*B. abortus*), attenuated strain 19 used for vaccination of cattle (11): obtained as a lyophilized preparation from R. I. T., Genval, Belgium; chlorite-oxidized oxyamylose (COAM): described in (12); mouse interferon was induced with NDV in L-929 cells and purified by ammonium sulfate precipitation (13); the resulting interferon preparation had a specific activity of circa 10^6 units/mg protein. Most of the compounds used in this study, viz. ESE, $(I)_n \cdot (C)_n$, $(I)_n \cdot (s^2C)_n$, f_2 dsRNA, NDV, *B. abortus*, and COAM, are well-established interferon inducers (for review, see (14)). They were all administered systemically as the provocative agent 24 hr after the skin had been sensitized with ESE.

Results and Discussion. Intracutaneous injection of ESE (50 μg) to nu/nu mice produced a mild local inflammation with perivascular accumulation of polymorphonuclear leukocytes (Fig. 1A). Macroscopically, this lesion appeared as a white spot of varying size and intensity. Quite often the white spot was rather faint (Fig. 2A). It generally subsided within 3–5 days. If the intracutaneous injection of ESE was

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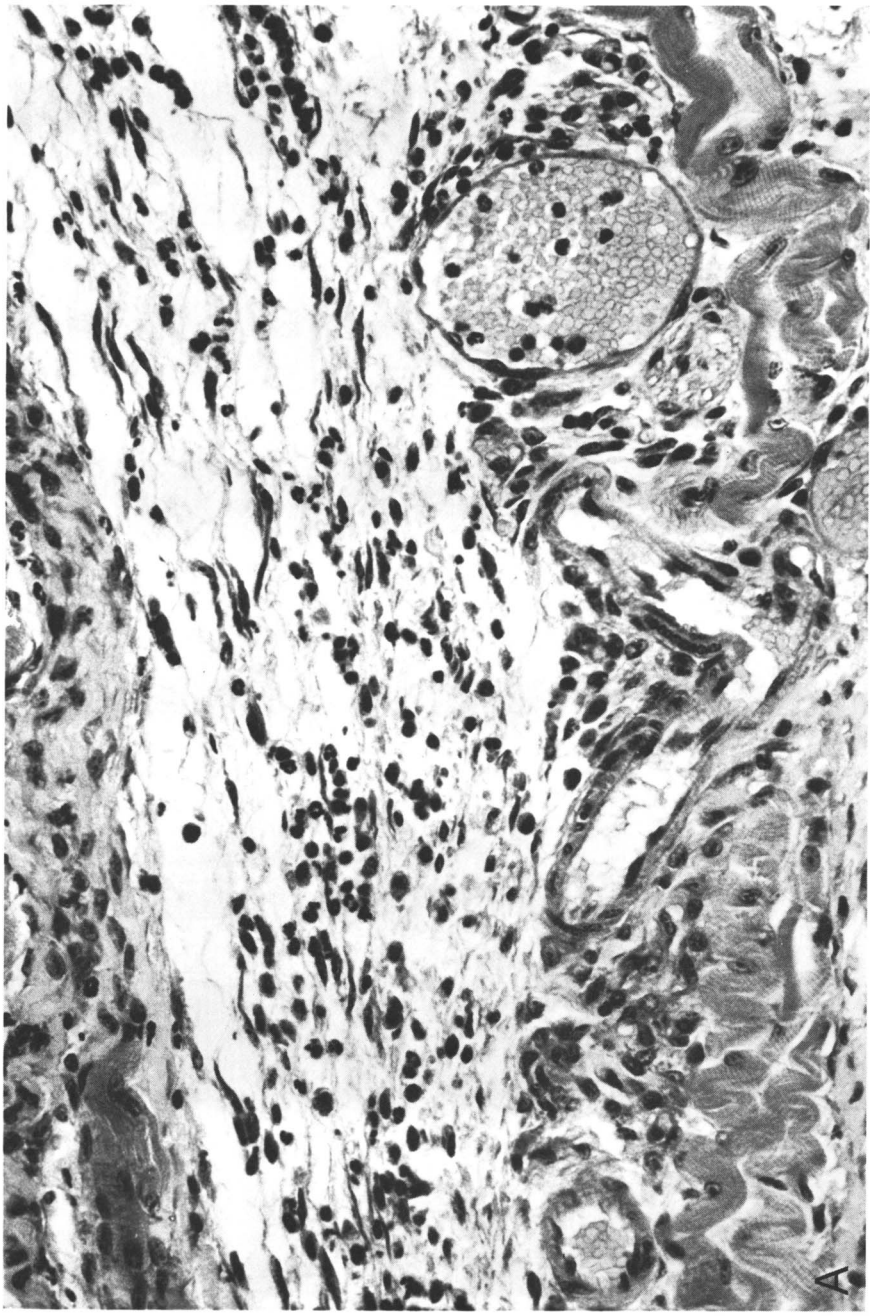
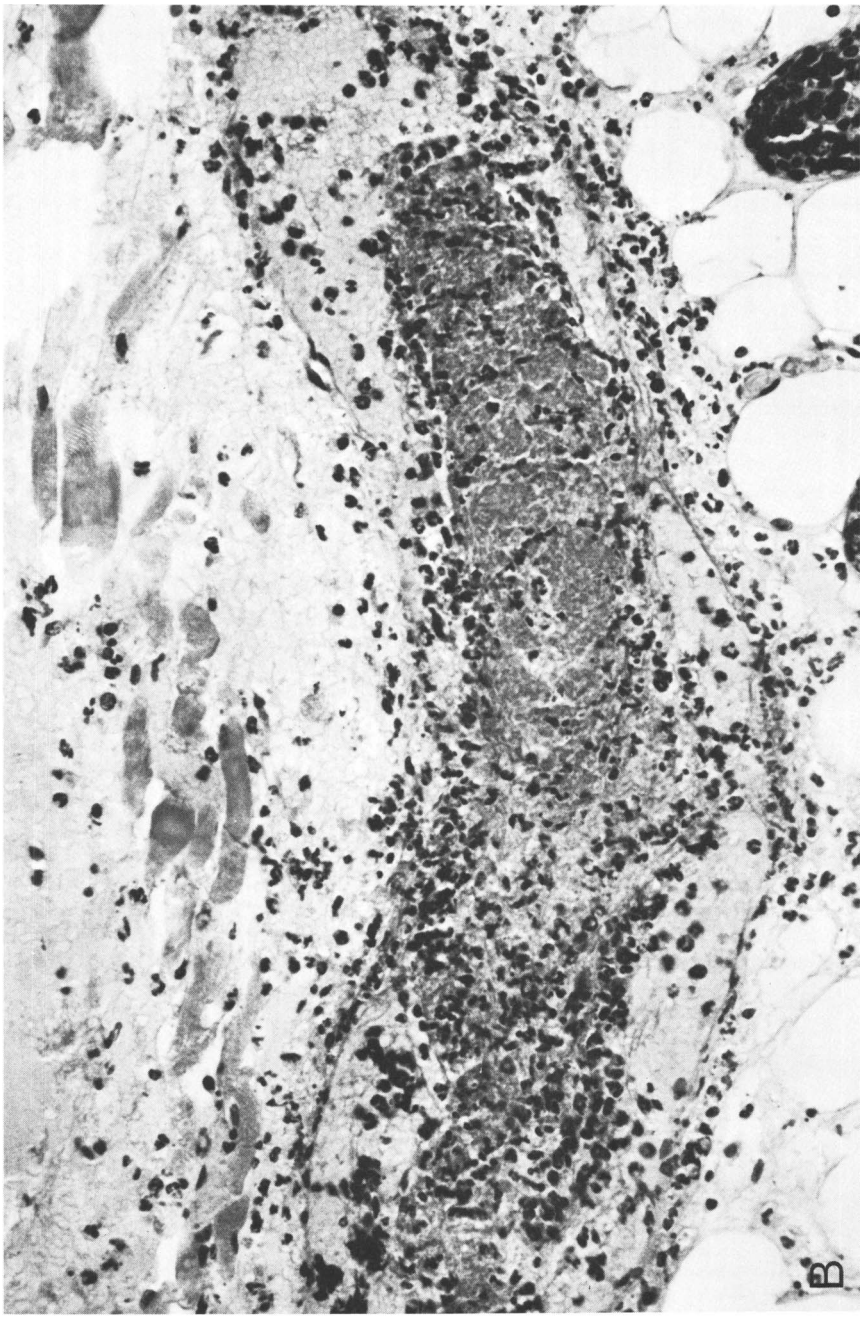


FIG. 1. Microscopic appearance of local Shwartzman reaction in athymic nude mice; histological section of skin: hematoxylin–erythrosin stain, ×400. (A) 48 hr after intracutaneous injection of ESE (50 μg/0.1 ml) and 24 hr after intravenous injection of PBS (0.2 ml). (B) 48 hr after intracutaneous injection of ESE (50 μg/0.1 ml) and 24 hr after intravenous injection of $(I)_n \cdot (C)_n$ (100 μg/0.2 ml). Pictures were taken from representative mice of the groups referred to in Table 1.



followed 24 hr later by an intravenous injection of PBS, there was obviously no change in the appearance of the reaction, but, if ESE or $(I)_n \cdot (C)_n$ were given as the provoker, then a black hemorrhagic lesion ensued at the site of the preparatory ESE injection (Fig. 2B). At some early stage (a few hours) after the provocative injection, small petechiae could be observed at the prepared skin site. Within the next hours the petechiae enlarged and coalesced to produce the characteristic hemorrhagic lesion depicted in Fig. 2B. Microscopically, the venules and capillaries of the affected skin showed occlusion of their lumen by fibrin thrombi and necrosis of their walls; they were surrounded by polymorphonuclear leukocytes, many of which were necrotic (Fig. 1B). The appearance of intravascular coagulation can be considered as pathognomonic for the Shwartzman reaction (1).

The Shwartzman reaction could be most clearly demonstrated with a preparatory dose of 50 μg of ESE and a provocative dose of 100 μg of $(I)_n \cdot (C)_n$, but a preparatory dose of 10 μg of ESE sufficed to sensitize the mice for the reaction and a provocative dose of 10 μg of $(I)_n \cdot (C)_n$ sufficed to elicit the reaction (Table 1). Unlike ESE, however, $(I)_n \cdot (C)_n$ did not act as a sensitizer (Table 1). Similarly, $(I)_n \cdot (C)_n$ failed to act as a sensitizer in rabbits (2), although some reports indicate that it may be able to do so (4).

In addition to $(I)_n \cdot (C)_n$, two double-stranded RNAs of natural origin, f_2 dsRNA and PC dsRNA, were also capable of provoking the Shwartzman reaction (Table 1). Despite its structural similarity to $(I)_n \cdot (C)_n$, $(I)_n \cdot (s^2C)_n$ failed to engender the reaction. Various other interferon inducers, viz. NDV, *B. abortus*, and COAM, were also explored as potential provokers of the Shwartzman phenomenon, but none of these compounds proved capable of pro-

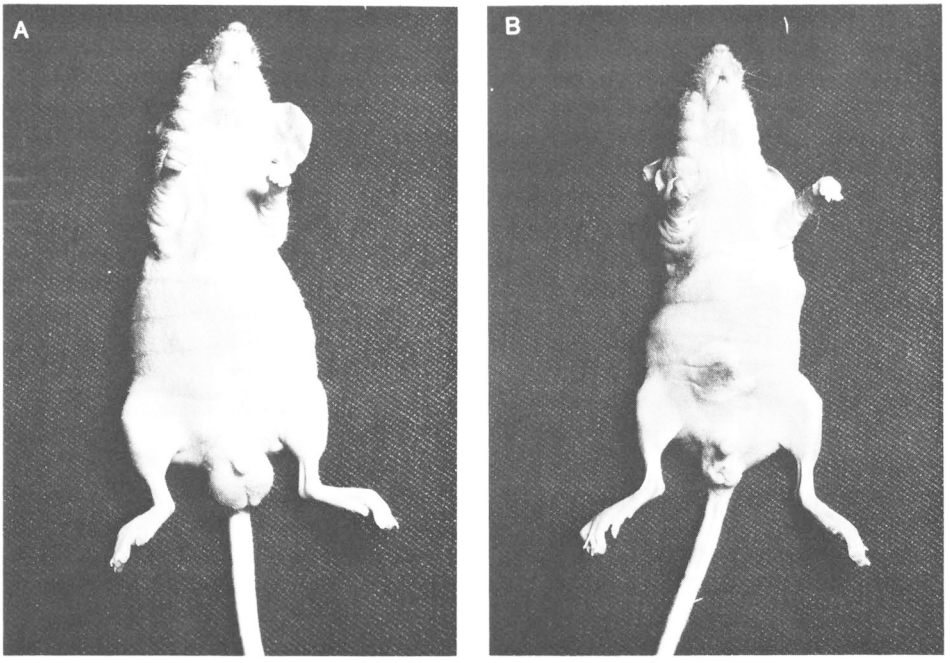


FIG. 2. Macroscopic appearance of local Shwartzman reaction in athymic nude mice. (A) Mouse taken 48 hr after intracutaneous injection of ESE (50 μg/0.1 ml) and 24 hr after intravenous injection of PBS (0.2 ml). Note faint white spot at the site of ESE injection. (B) Mouse taken 48 hr after intracutaneous injection of ESE (50 μg/0.1 ml) and 24 hr after intravenous injection of $(I)_n \cdot (C)_n$ (100 μg/0.2 ml). Note black hemorrhagic lesion, surrounded by white halo, at the site of ESE injection. Pictures were taken from representative mice of the groups referred to in Table 1.

TABLE I. LOCAL SHWARTZMAN REACTION IN ATHYMIC NUDE MICE

Preparatory injection[a]	Provocative injection[b]	Macroscopic appearance of reaction[c]
ESE (50 μg)[d]	PBS[d]	White spot[e]
ESE (50 μg)	ESE (100 μg)	Black hemorrhagic lesion (++)
ESE (50 μg)	$(I)_n \cdot (C)_n$ (100 μg)	Black hemorrhagic lesion (++)
ESE (50 μg)	$(I)_n \cdot (C)_n$ (10 μg)	Black hemorrhagic lesion (+)
ESE (50 μg)	$(I)_n \cdot (C)_n$ (1 μg)	White spot
ESE (10 μg)	$(I)_n \cdot (C)_n$ (100 μg)	Black hemorrhagic lesion (+)
ESE (2 μg)	$(I)_n \cdot (C)_n$ (100 μg)	No reaction
ESE (50 μg)	$(I)_n \cdot (s^2C)_n$ (100 μg)	White spot
ESE (50 μg)	F_2 dsRNA (100 μg)	Black hemorrhagic lesion (++)
ESE (50 μg)	PC dsRNA (100 μg)	Black hemorrhagic lesion (++)
ESE (50 μg)	NDV (10^8 egg ID_{50})	White spot
ESE (50 μg)	*B. abortus* (10^{10} bact.)	White spot
ESE (50 μg)	COAM (10 mg)	White spot
ESE (50 μg)	Mouse interferon (10^6 U)	White spot
$(I)_n \cdot (C)_n$ (50 μg)	$(I)_n \cdot (C)_n$ (100 μg)	No reaction
BSA (50 μg)	$(I)_n \cdot (C)_n$ (100 μg)	No reaction
PBS	$(I)_n \cdot (C)_n$ (100 μg)	No reaction
PBS	Mouse interferon (10^6 U)	No reaction

[a] Administered intracutaneously in a volume of 0.1 ml.

[b] Administered intravenously in a volume of 0.2 ml, 24 hr after preparatory injection; however, COAM was injected intraperitoneally and mouse interferon was injected either intravenously, intraperitoneally or subcutaneously (at the same site as the preparatory injection).

[c] At 24 hr after provocative injection; reaction is indicated as +, if lesion diameter < 0.5 cm, and ++, if lesion diameter ≥ 0.5 cm. Development of ++ lesion was often accompanied by mortality.

[d] Abbreviations of compounds as explained in the text; the indicated amounts refer to the individual doses per mouse.

[e] There were 10 mice per group and the reactions indicated for each group were those reactions that were considered representative for the group; thus, those reactions that were observed in at least 8, if not 9 or 10 of the 10 mice belonging to each particular group.

ducing the reaction (Table 1), and even interferon itself, when administered systemically or locally at a relatively high dose (10^6 U/mouse) failed to elicit a clear-cut Shwartzman response.

Serum interferon titers were determined at different times after the injection of the provocative agent. These measurements yielded the following peak interferon titers (times at which peak interferon levels were attained are indicated in parentheses): 10^4 U/ml (4–8 hr) for $(I)_n \cdot (C)_n$, < $10^{1.5}$ U/ml (4 hr) for $(I)_n \cdot (s^2C)_n$, $10^{3.5}$ U/ml (4–8 hr) for f_2 dsRNA, $10^{4.5}$ U/ml (8–12 hr) for NDV, $10^{2.9}$ U/ml (4–6 hr) for *B. abortus*, and $10^{2.6}$ U/ml (16 hr) for COAM. These results extend the initial observations on interferon induction by NDV in athymic nude mice by Pantelouris and Pringle (15) to a variety of interferon inducers other than NDV. It is remarkable that $(I)_n \cdot (s^2C)_n$ failed to stimulate interferon production in the nude mouse, although $(I)_n \cdot (s^2C)_n$ is as effective as $(I)_n \cdot (C)_n$ in inducing interferon in rabbits (8), dogs (8), and human cells (16). Since the lack of interferon-inducing ability of $(I)_n \cdot (s^2C)_n$ was matched by its failure to provoke the Shwartzman reaction, one could suspect an intermediary role of interferon in the pathogenesis of the Shwartzman reaction. However, this assumption was not borne out by our findings that NDV, in spite of its high interferon-inducing potency in the nude mouse, and interferon itself (produced *in vitro* with NDV as the inducer) failed to act as provokers of the Shwartzman reaction. It would appear, therefore, that the ability of endotoxin, $(I)_n \cdot (C)_n$, f_2 dsRNA and PC dsRNA to provoke the Shwartzman reaction in nude mice is based on the activation of some host mechanism(s) and/or the induction of some products other than interferon. At first glance, it is rather sur-

prising that double-stranded RNA and bacterial endotoxin, despite their structural dissimilarity, could switch on the same reaction(s). It should be emphasized, however, that there are many similarities in the biological effects of these substances, i.e., both endotoxin and dsRNA are pyrogenic (17) and embryotoxic (18), affect liver metabolism (19), stimulate antibody formation (4), and increase host resistance to viral, fungal, bacterial, and protozoal infections (as reviewed in (14)).

Attempts have also been made to establish a local Shwartzman reaction in laboratory animals other than nu/nu mice. The experimental procedure generally consisted of an intracutaneous injection of 50 γ ESE followed 24 hr later by an intravenous injection of $(I)_n \cdot (C)_n$ at a dose ranging from 100 μg to 1 mg per animal. In normal NMRI mice, guinea pigs, and athymic nude rats a faint white spot developed at the site of the ESE injection; this reaction subsided during the next few days, in spite of the provocative $(I)_n \cdot (C)_n$ injection. Hence, in these animals no Shwartzman reaction could be witnessed, at least not with $(I)_n \cdot (C)_n$ as the provocative agent. In rabbits which had been sensitized with ESE, a little erythema and edema developed at the ESE injection site; this reaction became more pronounced and evolved to an induration after the provocative $(I)_n \cdot (C)_n$ dose was given. Although the latter reactivity could qualify as a genuine Shwartzman phenomenon (2–4), the lesion had no hemorrhagic or necrotic character as classically postulated for the localized Shwartzman reaction (1).

Conclusion. Athymic nude mice appear to be an ideal animal model to demonstrate the local Shwartzman reaction. This may be related to the congenitally athymic state of the nude mice, their impaired T-lymphocyte response, their abnormal skin structure, or other peculiarities such as an increased macrophage activity or an aberrant responsiveness to endotoxin. Nude mice have indeed been shown to be highly resistant to infection by facultative intracellular bacteria by virtue of an enhanced bactericidal activity of their macrophages (20), and, unlike conventional mice, they do not produce a glucocorticoid antagonist in response to endotoxin (21).

The pathogenesis of both the localized and generalized Shwartzman reactions remains ill-defined. Both platelets and polymorphonuclear leukocytes, as well as complement factors, appear to be essential to the expression of the reaction ((22) and references cited therein). While some studies (22) suggest that the Shwartzman reaction represents a manifestation of immunologic injury, other studies (23, 24) refute this possibility. Whatever mechanism the Shwartzman reaction may be based upon, the nude mouse model provides a unique system to evaluate the role of T- and B-cell functions, and other host factors, in the pathogenesis of what has long since (25) been recognized as a most intriguing phenomenon.

Summary. Athymic nude mice appear to be an ideal animal model to demonstrate the local Shwartzman reaction; in these mice the reaction can be provoked by both endotoxin and double-stranded RNA.

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