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BEAL *ET AL.* REPLY—Davies and Roberts contend that quinolinic acid striatal lesions do not spare somatostatin (NADPH-diaphorase) neurons. We have attempted to resolve the discrepancy by carefully re-examining our own material. There is one major methodological difference between the two reports. Davies and Roberts examined regions containing the lesion cores in which there is almost total neuronal loss and marked gliosis as depicted in their Fig. 1 and the data given in Fig. 2*a*. They found no selective sparing of neurons within this region and our own findings are in agreement with that result. Since we believe that sparing of somatostatin neurons is relative and not absolute, we make our counts in regions posterior to the lesion, where there is a depletion of total Nissl-stained neurons in the range of 50%. Within this region we find significant increases in NADPH-diaphorase neurons relative to acetylcholinesterase neurons. We have further examined this issue by counting numbers of NADPH-diaphorase neurons relative to the total number of Nissl-stained neurons. NADPH-diaphorase neurons are significantly increased as a percentage of the total neuronal population in this area. These results are consistent with the findings of Choi and colleagues who showed that quinolinic acid results in relative, but not absolute, sparing of NADPH-diaphorase neurons in cortical cell cultures (*Science* **234**, 73-76; 1986).

Davies and Roberts do not take issue with our neurochemical data. We have replicated these results in numerous subsequent experiments and they have been replicated by others (Nemeroff, Kitt, Dissette and Schwarcz, personal communication). Davies and Roberts explain these results by asserting that somatostatin may be contained in striatal afferents. However, this cannot explain differential effects of kainic acid and quinolinic acid on somatostatin levels, since both produce axon-sparing lesions.

It is likely that quinolinic acid lesions produce sparing of both acetylcholinesterase and NADPH-diaphorase neurons relative to other neuronal populations. We have recently found that acetylcholinesterase neurons are also spared in Huntington's disease (Ferrante *et al.*, *Brain Res.* **411**, 162-166, 1987). These findings taken as a whole strengthen the validity of our original observations that quinolinic acid lesions are an animal model of Huntington's disease.

M. F. BEAL
N. W. KOWALL
J. B. MARTIN

Department of Neurology,
Massachusetts General Hospital
and Harvard Medical School,
Boston,
Massachusetts 02114, USA

The third component of complement (C3) is responsible for the intracellular survival of *Leishmania major*

David M. Mosser & Paul J. Edelson

Department of Pediatrics, Division of Infectious Diseases and Immunology, New York Hospital and Cornell University Medical College, New York, New York, USA

Leishmania **are obligate intracellular parasites of mononuclear phagocytes. We[1,2] and others[3] have shown that the promastigote form of all species of leishmania activates complement from non-immune serum and that this activation can result in parasite lysis. This work, as well as earlier *in vivo* studies[4], suggested that complement is an important component of host defence against leishmaniasis. We now present evidence that parasite complement fixation, in addition to increasing parasite phagocytosis[5,6], is required for the intracellular survival of leishmania in macrophages. We specifically show a strong correlation between parasite C3 fixation and intracellular survival. We attribute this survival, in part, to a decrease in the magnitude of the macrophage respiratory burst which is triggered by complement-coated, as opposed to uncoated, parasites.**

Labelled *Leishmania major* promastigotes are added to monolayers and the number of intracellular organisms is determined at either 24 or 48 h and compared to the number of parasites which were phagocytized during the first hour. The number of organisms associated with the monolayer at one hour is determined with a parasite radiobinding assay, which measures the total number of macrophage-associated organisms[1]. In separate assays, using two independent methods, we have determined that more than 85% of organisms in all groups studied are internalized during this initial incubation period.

Over a wide range of parasite inputs, in the absence of serum, resident mouse macrophages kill over 95% of the ingested promastigotes, but when fresh non-immune serum is included, there is more than a 10-fold enhancement in survival (Fig. 1). Because of the increase in binding due to serum (opsonization) the average number of parasites bound per macrophage is higher, which might account for this increased survival. We therefore measured intracellular survival after the parasite input had been adjusted by two-thirds to three-quarters to achieve similar binding in the presence or absence of serum. Table 1 shows that ~95% of the 3.1 organisms bound per macrophage in the absence of serum are killed. In addition, fewer than 5%

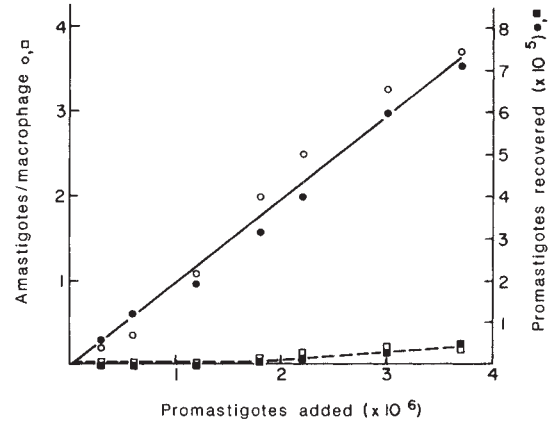

Fig. 1 The intracellular survival of *L. major* in resident murine macrophages. Increasing numbers of promastigotes were added in the presence (circles) or absence (squares) of 4% mouse C5D serum. Left axis, average number of morphologically intact amastigotes per macrophages counted on acridine orange-stained monolayers 48 h after infection (open symbols). The right axis indicates the number of viable organisms present per monolayer measured by the parasite reculture method which involves counting the number of promastigotes which tranform from amastigotes as described[7]. Amastigotes are released from hypotonically lysed monolayers 24 h after infection (closed symbols). In separate experiments we have determined that the number of intracellular organisms remains constant from 24-48 h by this assay.

of the macrophages contain organisms at 48 h (data not shown). In the presence of non-immune serum, macrophages which bind 4.1 parasites per cell support an average of 1.1 organisms/cell at 48 h. This enhancement does not depend on the continuous presence of fresh serum, as parasites which are briefly preopsonized with serum and then washed before their addition to monolayers survive as well as those ingested in the continuous presence of serum (Table 1 and Fig. 2). Heated serum, or serum treated with EDTA , does not support intracellular survival, suggesting a role for complement, but serum lacking C5 or C8 are fully competent, ruling out a requirement for the terminal complement components (Table 1). Normal human serum specifically depleted of C3 (C3D) was obtained by exposing serum to a monospecific antibody to C3 (Dako, California) for 1 h followed by antibody removal with Pansorbin (Calbiochem, California). C3-depleted serum cannot opsonize leishmania for enhanced parasite uptake and is unable to mediate intracellular survial. The addition of 250 U of purified C3 (Cordis, Miami, Florida) to this serum (C3D+C3) partially restores parasite intracellular survival (Table 1).

Initially, parasite opsonizations were performed for 7 min in serum which was deficient in one of the late complement components, to avoid parasite lysis. These opsonized parasites are fully viable and, when placed back into culture, show growth curves which are identical to untreated organisms. A slightly shorter exposure (5 min), also allows the use of intact human serum without parasite lysis. This brief preopsonization in intact serum results in the deposition of 4×10^4 molecules of C3 per parasite (determined using iodinated C3 as described previously[7]) and is sufficient to enhance parasite intracellular survival optimally. This suggests that complement fixation may be beneficial to the parasite *in vivo* even in normal hosts with an intact lytic complement pathway.

That this brief exposure to serum was sufficient to deposit C3b on the surface of the parsites (Table 1) was also shown by indirect immunofluorescence using a monoclonal antibody specific to C3b[8] generously provided by P. J. Lachmann. All opsonizing conditions which allow enhanced parasite survival result in C3 deposition on the parasite surface (C8D, C5D, NHS, C3D+C3). Fluorescence is uniform throughout the parasite population with all parasites staining positive for bound C3. All conditions which do not result in C3 deposition fail to mediate intracellular survival (Table 1).

Because Leishmania are sensitive to superoxide and other toxic oxygen products[9,10], we compared the respiratory burst triggered by parasites in the presence or absence of serum. By measuring the respiratory burst during the phagocytosis of para-

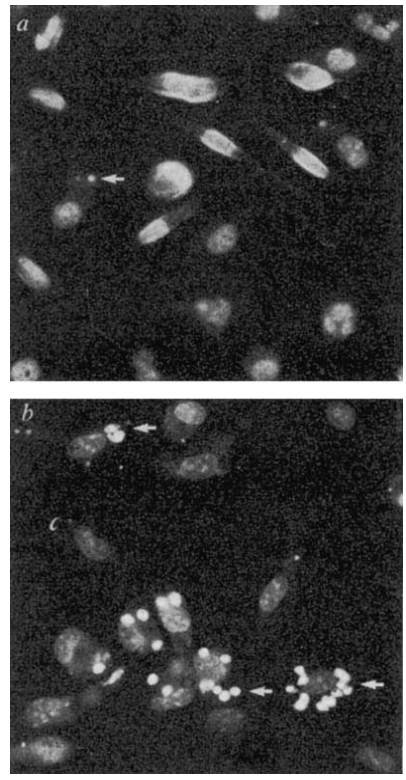

Fig. 2 Acridine orange (AO) staining of macrophage monolayers infected 48 h earlier with *Leishmania major* promastigotes which were preincubated with *a*, saline or *b*, C5-deficient (C5D) mouse serum. Parasites were resuspended to a concentration of 2×10^8 ml^{-1} in phagocytosis buffer[2] and exposed to either saline or 7% C5D for 7 min. Parasites were then washed twice and added to macrophages for 45 min at 36 °C. To achieve similar numbers of parasites ingested, 3×10^6 parasites were added in *a* and 1×10^6 organisms were added in *b*. Macrophages on coverslips were then thoroughly washed, placed in fresh wells, and washed again at 2 and 12 h. At 48 h cells were washed in Hanks balanced salt solution (HBSS), stained for 30 s in a 1% AO solution in water, diluted 1:50,000 in HBSS. Monolayers were then washed and fixed in 2% formalin and examined under a fluorescence photomicroscope. Arrows point to intact intracellular organisms. Minimal cytoplasmic staining causes some intracellular organisms to appear to be extracellular. Acridine orange staining is performed at 48 h because heavily infected macrophages sometimes require a full day to degrade killed parasites completely.

Table 1 The interaction of *Leishmania major* promastigotes with murine macrophages

	Phagocytosis*	*n*	Viability†	*n*	Morphology‡	*n*	C3 bound§
Serum independent	3.1±0.29	8	0.19±0.10	6	0.16±0.05	6	−
4% Human C8D	4.1±0.33	7	1.83±0.34	3	1.10±0.1	5	+
Preopsonized¶							
Mouse C5D	4.5±0.71	5	1.55±0.19	4	1.71±0.27	3	+
Normal human	3.3±0.51	5	ND		1.10±0.31	2	+
HI−HS*	5.1±1.9	4	0.15±0.05	2	0.11±0.10	3	−
HS−EDTA**	3.2±0.35	3	0.20	1	0.09±0.10	2	−
HS−C3D	2.9±0.32	2	0.25±0.07	2	0.20±0.14	2	−
HS−C3D+C3††	2.6±0.19	2	0.87±0.31	2	0.63	1	+
Heat killed Ltm‡‡	3	2	0	1	0	2	

* Number of organisms phagocytosed at one hour, determined by the radiolabelled parasite binding assay, as described[1].
† Number of viable parasites present at 24 h, determined by the reculture method[7].
‡ Average number of intact intracellular organisms per macrophage at 48 h, determined by the acridine orange technique[12].
§ The presence of bound C3 was determined by immunofluorescence microscopy as described[5] using a monoclonal antibody to C3 (ref. 8).
‖ Mean ± standard error.
¶ Parasites were preopsonized in 7% serum for 7 min with the exception of those exposed to normal human serum (NHS) which were preopsonized for 5 min.
⁂ Human serum was heat-inactivated for 2 h at 56 °C.
** Parasites were exposed to human serum containing 9 mM EDTA.
†† C3-depleted serum to which 250 U of C3 (Cordis) was added.
‡‡ Promastigotes were treated at 60 °C for 30 min.

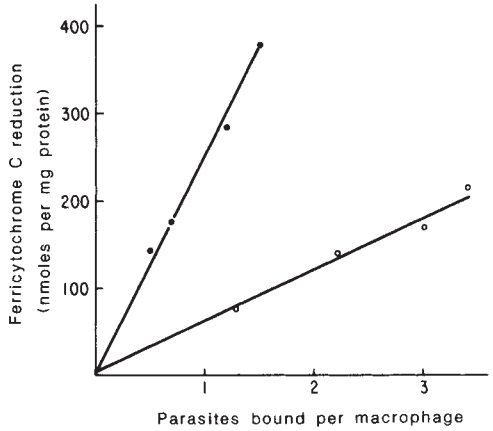

Fig. 3 The macrophage respiratory burst triggered during the phagocytosis of opsonized (○) or unopsonized (●) *Leishmania major* promastigotes, measured by the reduction of ferricytochrome C, as described by Johnston[13]. Parasites were opsonized for 7 min in 7% human C8D. The ordinate is the number of nmoles of cytochrome C reduced h^{-1} per mg protein. The abscissa is the average number of radiolabelled organisms bound per macrophage, determined by the parasite radiobinding assay[1].

sites labelled with tritiated uracil, we could simultaneously measure the number of parasites bound and the respiratory burst they trigger on the same coverslip. Figure 3 shows that with either opsonized or unopsonized parasites, the magnitude of the respiratory burst increases in proportion to the number of parasites bound. C3-opsonized promastigotes, however, are poorer at stimulating a respiratory burst than are unopsonized organisms (Fig. 3). Equal numbers of parasites bound to the macrophage in the absence (2.2 ± 0.19, $n = 5$) or presence of serum (2.2 ± 0.29) trigger respiratory bursts of 178 ± 38 nmol O_2 per mg protein and 63 ± 12 respectively, a decrease of 65%. The respiratory burst triggered by opsonized zymosan, measured in parallel experiments, is 207 nmol O_2 per mg protein. The diminished production of superoxide measured in the presence of serum is not due to a non-specific protein quench as the burst is not diminished in the presence of 4% heat-inactivated serum.

We do not believe that serum immunoglobulins, which may be cross-reactive with the parasite[2] are responsible for enhanced intracellular survival. The brief time of preopsonization which we chose, 5–7 min, is not sufficient for the fixation of detectable amounts of immunoglobulin on the parasite's surface, by immunofluorescence microscopy (data not shown). Further, serum exposed to Protein A alone, performed as a control for the C3 depletion experiments, continues to mediate parasite survival. Finally, the addition of heat-inactivated antiserum to *L. major*, raised in rabbits, does not mediate parasite survival.

We therefore conclude that parasite-bound C3 plays a central role in the parasitism of macrophages by leishmania. Our previous work[1,2] and that of others[3] demonstrated that all species of leishmania activate complement. We have also shown that this activation results in an enhanced parasite uptake by macrophages via their complement receptors[5]. The present work demonstrates that even when equal numbers of *L. major* promastigotes are bound, the intracellular fate of C3-coated organisms is significantly different from that of non-opsonized organsims. Only those parasites with C3 on their surface establish a successful intracellular parasitism. We relate this successful parasitism, in part, to a decreased triggering of the respiratory burst by serum-opsonized organisms. Others have shown that macrophage complement receptors fail to trigger a respiratory burst[11].

This suggests that complement activation by leishmania and perhaps other organisms may be a common mechanism seized upon by intracellular parasites to ensure their successful entry into phagocytic cells which bear complement receptors.

This work was supported in part by the US National Institutes of Health.

Received 12 March; accepted 7 April 1987.

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Genetic rescue of inviable hybrids between *Drosophila melanogaster* and its sibling species

Pierre Hutter & Michael Ashburner

Department of Genetics, University of Cambridge, Cambridge CB2 3EH, UK

Post-mating mechanisms are central to the establishment of reproductive isolation between different, but closely related, species. Post-mating isolation mechanisms include hybrid breakdown, hybrid sterility and hybrid lethality[1,2] and may, in some cases, be reinforced by pre-mating mechanisms such as ethological differentiation. In the *Drosophila melanogaster* species sub-group post-mating reproductive isolation is ensured by both the inviability and the sterility of hybrids[3]. For example when *D. melanogaster* females are crossed to *D. simulans* males the hybrid progeny are normally all female; the hybrid males die as third instar larvae[4–6]. The viable hybrid females are totally sterile[4,5]. Little is known of the genetic basis for either hybrid sterility or hybrid inviability, although Coyne and others[7,8] have begun a genetic analysis of the sterility of hybrids within this species sub-group. We have discovered a single gene difference that rescues the otherwise inviable male hybrids from the cross between *D. melanogaster* females and males of its three closest relatives. The study of this locus may shed light on the genetic control of both speciation and development.

The *D. melanogaster* species sub-group consists of eight sibling species, six of which are endemic to the Afrotropical region or islands of the Indian Ocean. Four of these species are very closely related; they can only be reliably distinguished by their male genitalia and they will hybridize under laboratory conditions. These are the two cosmopolitan species *D. melanogaster* and *D. simulans*, *D. mauritiana*, endemic to the island of Mauritius, and *D. sechellia*, endemic to some islands of the Seychelles archipelago[9]. When *D. melanogaster* females are mated to males of the other species all the viable adult hybrids are sterile females. Males die as third instar larvae[4,9,10]. Occasional surviving males are seen, these are X0 and result from the fertilization of nullo-X *D. melanogaster* eggs (the products of primary non-disjunction) by X-bearing sperm[4]. Watanabe[11] discovered an unusual strain (*K18*) of *D. simulans* from Japan that, when crossed to *D. melanogaster*, gave hybrid progeny of both sexes. The factor responsible for the rescue of the inviable hybrid class (*Lhr*) was mapped to chromosome arm 2R. Unfortunately, the paucity of genetic markers and aberrations in *D. simulans* has made it difficult to study this gene further. For this reason we screened 63 natural populations of *D. melanogaster* for an analogous mutation in this species. Females from these strains were crossed to males from a panel