

# Murine Immune Responses and Immunization Against *Polyplax serrata* (Anoplura: Polyplacidae)

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J. Med. Entomol. 27(6): 1002-1007 (1990)

**ABSTRACT** Mice with restricted grooming capabilities were infested with the solenophagous louse, *Polyplax serrata* (Burmeister). Louse burdens on Cox/Swiss and C3H/HeSN mice increased for approximately 1 mo, reaching burden/host weight ratios of 1.14 and 1.26 mg/g, respectively, followed by a steady decline. Fifty days after initial ectoparasite contact, both strains were resistant to lice. Resistance was anamnestic, lasting several months with second infestation weights reduced by 98 and 78% on Cox/Swiss and C3H/HeSN, respectively. Furthermore, mice were systemically resistant because infestations on naive body sites of resistant hosts were reduced by 59%. Host resistance was associated with the development of antilouse immune responses. After the first week of a primary infestation, the draining lymph nodes contained cells that proliferated in vitro to louse antigens. Skin responses to louse antigens were also detected: (1) delayed, (2) immediate and delayed, and (3) no significant reactivity on days 19, 34, and 54, respectively. The presence of systemic antilouse responses provided an immunologic basis for immunization against lice. Intradermal injections of soluble louse components reduced primary infestation weights by 62%. Immunized mice had immediate and delayed skin responses containing an inflammatory infiltrate 1 wk following immunization. This study, using the natural host of *P. serrata*, demonstrates an inducible, anamnestic immune component in louse resistance.

**KEY WORDS** Insecta, *Polyplax serrata*, mice, immunity

MICE USED FOR RABIES RESEARCH in the 1960s developed substantial burdens of *Polyplax serrata* (Burmeister) on the nape area because diseased mice lost limb function, which reduced grooming. Subsequently, host resistance to lice was studied in healthy mice disabled by a hind-toe amputation (Bell et al. 1962). Louse burdens peaked after 4 wk, followed by a steady decline with hosts resistant to reinfestation. Host resistance was considered a local skin phenomenon because hosts were not resistant to a second infestation at a naive site created by a second amputation (Bell et al. 1966). Second, skin grafts from resistant mice to athymic recipients transferred resistance only if taken from the infested area (Bell et al. 1982). Histologically, the infestation site contained various circulating and resident cells whose numbers correlated with resistance (Nelson et al. 1972, 1979).

These studies show that resistance is expressed at the infestation site, but they did not establish if louse resistance originated in the skin or elsewhere. Therefore, we re-examined anamnestic resistance on naive sites and determined if hosts developed systemic skin responses to louse antigens. Lym-

phoid tissues were tested for louse-reactive cells in vitro and a simple immunization procedure was attempted to induce louse resistance in mice.

## Materials and Methods

**Infestations.** Solenophagous lice, *Polyplax serrata* (Burmeister), were received pathogen-free from the Rocky Mountain Laboratory, Hamilton, Mont., and maintained on Cox/Swiss mice (Laboratory Supply Company, Indianapolis, Ind.). Scanning electron micrographs confirmed our louse colony as *P. serrata* based on the morphology of tergites, paratergites, legs, head, and dorsal principal seta. Cox/Swiss and C3H/HeSN mice (Jackson, Laboratory, Bar Harbor, Maine) were raised in our facility with breeding pairs replaced from company stocks to maintain genetic lines. Hind-toe amputations at the metatarsal joint were performed at birth, and the mice were used at 6-8 wk of age. Disabled mice were infested by placing 5 mg of lice on the shoulder. The challenge burden consisted of approximately 250-300 nymphal and adult stages. Infested mice were caged individually to prevent grooming and were maintained in a temperature-controlled room at 22°C with a photoperiod of 12:12 (L:D). Infested mice were killed by cervical dislocation, supported by wire mesh over weight boats, chilled at 4°C for 1 h, then

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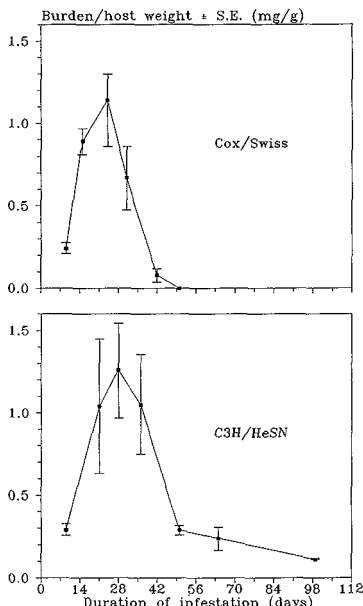


Fig. 1. Growth curves for *P. serrata* on murine hosts reported as a burden-to-host-weight ratio (mg/g). Each point was determined from eight Cox/Swiss or four C3H/HeSN mice ( $\bar{x} \pm \text{SE}$ ).

placed above a 37°C water bath. Lice migrated to the warmth and were collected in weigh boats. Louse growth was quantitated by comparing burden with host body weight (milligram per gram).

**Systemic Resistance.** Double hind-toe amputations on Cox/Swiss neonates restricted nape and shoulder grooming. At 6 wk, the animal's right shoulder was shaved and the left shoulder was infested. The shaved area limited the infestation to the left shoulder because lice use the hair shaft for mobility, feeding, and egg attachment (Nuttall 1918). The shaved area covered a block (approximately 4 by 7 cm) which started at the ear and bordered the middorsal line. Frequent observations confirmed that the hairless site was louse-free. At the conclusion of a primary infestation, mice were allowed to groom each other, which freed them of residual louse burdens and restored coat condition on both shoulders. This louse-free period lasted 30 or 96 d and was followed with a second infestation on both shoulders in which the right shoulder served as a naive skin site.

**Louse Antigen.** Lice were stored at -80°C and served as a common source for preparation of soluble louse components (SLCs). Lice were disrupted in phosphate-buffered saline (PBS), pH 7.2, with a glass tissue grinder, followed by ultrasonication of six 20-s bursts with a microprobe set at 90 W (Heat Systems-Ultrasonics Inc., Plainview, N.Y.). Whole-lice sonicates were centrifuged at 12,000  $\times$  g for 30 min; the supernatant contained the SLC. All preparations of SLC were free of viable bacteria when plated on blood agar medium.

**Skin Tests and Histology.** Ears were skin-tested with an intradermal injection of SLC, 10  $\mu$ g of protein in 10  $\mu$ l PBS, using a 27-gauge needle. The left ear received a control injection of 10  $\mu$ g of bovine serum albumin (BSA). Ear thickness measurements were obtained with an engineer's micrometer at 6, 24, 48, 72, and 96 h after antigen injection. Naive mice were used for background controls (swelling subtracted). Histological procedures were conducted according to the method of Mallory (1968). Briefly, 3-mm tissue specimens from the ear test sites were fixed for 24 h at 4°C in 2% paraformaldehyde + 5% glutaraldehyde, dehydrated in graded alcohol, and embedded in paraffin. Tissue sections (5  $\mu$ m) were deparaffinated and stained with hematoxylin-eosin.

**Proliferation Assay.** Lymphoid cells from the draining lymph nodes or spleens of five C3H/HeSN mice were pooled and adjusted to  $5 \times 10^6$  cells/ml of RPMI 1640 media. Flat-bottom microculture plates were filled with 0.1 ml of cells and incubated for 96 h in a 5% CO<sub>2</sub> atmosphere with SLC at 0.5, 50, 250, and 500  $\mu$ g/ml. Proliferation was determined by adding one  $\mu$ Ci of [methyl-<sup>3</sup>H]-thymidine (New England Nuclear, Boston, Mass.; specific activity 6.7 Ci/mM) for the last 16 h of the incubation. Cells were harvested on glass filters, dried, and counted in a liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.).

**Immunization.** Cox/Swiss mice were divided into three groups, eight mice per group, and injected with PBS, BSA, or SLC each day for 6 d. Adjuvants were not used, and the injections near the base of the tail were outside of the infestation area. Each injection contained 100  $\mu$ g of protein in 100  $\mu$ l of PBS. One week after the final injection, immunized mice were either infested or skin-tested with SLC.

**Statistical Analysis.** The Student *t* (two-sample) test was used to compare data.

## Results

Two murine strains with different population gene pools served as hosts for generating louse growth curves. Outbred Cox/Swiss mice represent a heterogenic population, whereas C3H/HeSN are syngenic. C3H/HeSN were used to eliminate alloreactive immune activity during *in vitro* assays. Both strains developed resistance after 3-4 wk of exposure (Fig. 1). Cox/Swiss mice experienced maximum burdens of 34 mg or burden/host weight

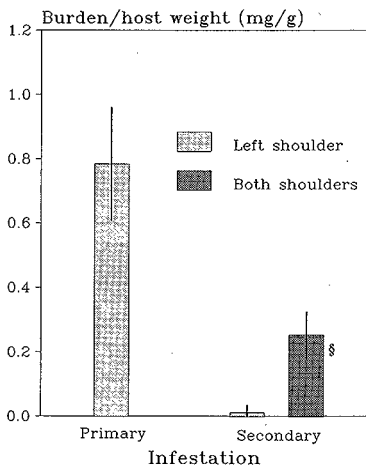


Fig. 2. A primary *P. serrata* infestation, restricted to the left shoulder, induced resistance on both shoulders. Burden-to-host-weight ratios were determined on day 24 from eight mice ( $\bar{x} \pm SE$ ), §, compared with primary infestation on left shoulder ( $P < 0.01$ ).

ratios of 1.14 mg/g on day 24 of the infestation and fell to 0 by day 50. C3H/HeSN developed peak burdens of 29 mg or burden/host weight ratios of 1.26 mg/g on day 28, followed by a steady decline, but they did not eliminate the infestation after 98 d. The data reported were collected in a single representative experiment and demonstrates the intensity and duration of the burdens collected on Cox/Swiss and C3H/HeSN throughout these studies. The 24- and 28-d infestations were selected as the time points to collect louse burdens in subsequent experiments designed to assess systemic resistance.

To test if mice had acquired systemic resistance, primary infestations were restricted to the left shoulder by shaving, followed by a second infestation on both the left and right (naive) shoulders (Fig. 2). A second infestation on the same site and initiated 30 d after the primary infestation was significantly reduced with a burden/host weight ratio of  $0.01 \pm 0.01$  mg/g compared with the primary infestation ( $0.80 \pm 0.22$  mg/g;  $P < 0.005$ ). Furthermore, 30 d after a primary infestation on the left shoulder, a second infestation on both shoulders was uniformly distributed and significantly smaller (burden/host weight ratios of  $0.33 \pm 0.09$  mg/g;  $P < 0.01$ ). A repeat of this experiment confirmed these data and showed that the duration of host resistance was maintained when the louse-free period between infestations was extended to 96 d

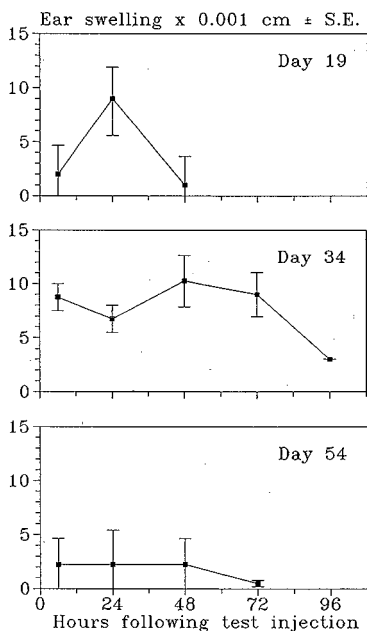


Fig. 3. Skin responses to soluble louse components after *P. serrata* infestations of 19, 34, and 54 d. Naive mice were used for background controls, and swelling was subtracted from these data. Each point was determined from four C3H/HeSN mice ( $\bar{x} \pm SE$ ).

(data not shown). These data demonstrate that mice acquire systemic, anamnestic resistance which is not dependent upon continuous louse exposure but is maintained through louse-free periods.

Skin tests were conducted on mice with 19-, 34-, and 54-d infestations, time points representing different stages of the infestation (Fig. 3). Skin tests with SLC produced the following swelling pattern: on day 19, delayed only; on day 34, immediate and delayed; and on day 54, no significant skin swelling. Skin-swelling responses were specific because only exposed mice responded to SLC, and exposed mice did not respond to a heterologous protein (BSA) (data not shown).

Lymph node cells pooled from five mice proliferated in culture when incubated with SLC (Fig. 4). The proliferating lymphoid cells, detected on days 8 and 22, responded to increasing doses of SLC. At a dose of 250  $\mu$ g SLC/ml, cells from infested mice incorporated  $26,238 \pm 2,033$  and  $76,951$

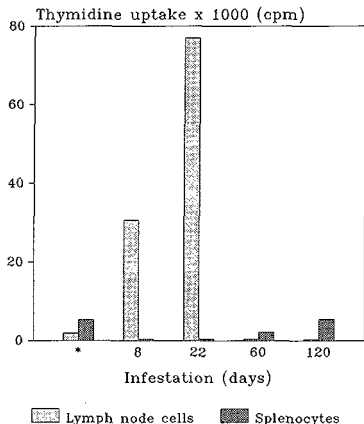


Fig. 4. Proliferative responses of lymphoid cells to soluble louse components, 250  $\mu$ g/ml. \*, naive mice.

$\pm$  5,122 cpm of thymidine on days 8 and 22, respectively; background levels for naive mice were 1,865  $\pm$  208. Thymidine uptake indicates that the louse infestation activated cells in draining lymphoid node tissue. Splenocytes, in contrast, did not respond at any time point tested through day 120.

Louse resistance was artificially induced in two separate experiments by immunization with six daily injections of SLC. Subcutaneous immunization reduced burden/host weight ratios by 62% compared with buffer controls ( $P < 0.01$ ) (Fig. 5). For a comparison, the burdens on BSA-immunized mice were smaller but not significantly different from PBS controls. Second infestations on nonimmunized hosts were reduced by 94% ( $P < 0.0001$ ).

One week after immunization, mice had early and delayed SLC skin tests (Fig. 6). Mice immunized with BSA had small positive reactions with SLC (0.003  $\pm$  0.002 cm of ear swelling) (data not shown), which may explain their smaller burdens. Tissue biopsies from the SLC test site of SLC-immunized mice contained infiltrating cells compared with few infiltrating cells in controls (Fig. 7). These data demonstrate that SLC immunization induces resistance in mice by immune-mediated inflammation of the skin.

#### Discussion

The murine strains used in these studies developed systemic, anamnestic resistance to lice. Our data show that host responses were not restricted to the infestation site but were expressed systemically. The systemic nature of louse resistance was

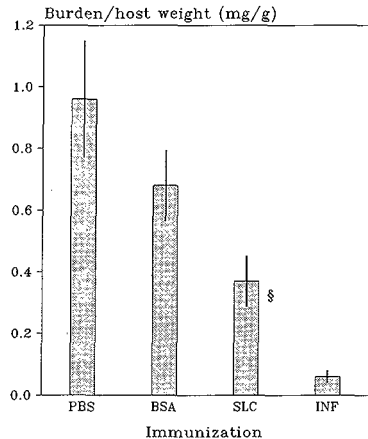


Fig. 5. Immunization with soluble louse components reduces *P. serrata* burden-to-host-weight ratios. Groups of eight mice each received injections of phosphate-buffered saline (PBS), bovine serum albumin (BSA), and soluble louse components (SLC). INF, second infestation. \$, SLC compared with PBS ( $P < 0.01$ ). BSA compared with PBS was not significant.

demonstrated in naive skin sites, then was further characterized by elicitation of skin reactions away from the infestation site in the ears and proliferative responses in lymph nodes. Finally, resistance was induced by immunizing mice with the soluble components of disrupted lice.

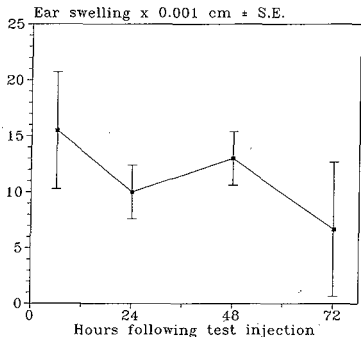


Fig. 6. Skin responses to soluble louse components 1 wk after immunization. Each point represents the mean ear swelling of four mice ( $\bar{x} \pm$  SE).

Skin responses to lice develop during the infestation. The onset and intensity of these skin reactions correlate with the onset of host resistance, which is marked by declining louse burdens. Tolerance to SLC antigens late in the infestation (by day 54) indicates that the host is regulating the intensity and duration of immune responsiveness. Immunologic tolerance may explain the residual burdens on C3H/HeSN mice in these studies and the phenomenon of carrier animals in domestic cattle (Nelson et al. 1970). A changing sequence of skin responses is not unique to the louse-mouse relationship; it was also reported in humans against *Aedes aegypti* (L.) (Mellanby 1946) and in guinea pigs to the cat flea, *Ctenocephalides felis* (Bouché) (Benjamini et al. 1960a,b). Furthermore, systemic skin responses to lice were reported in human case studies. Experimental infestations on the forearm with *Pediculus corporis* produced a systemic response on the upper body characterized by macular erythematous skin rashes (Hirshfelder & Moore 1919).

Before the appearance of murine skin reactivity on day 19, lymph node cells had already been activated by louse antigens as early as the eighth day of a primary infestation. These proliferative responses are the first indication that either T or B antilouse lymphocytes are being activated because both cell populations can proliferate in response to antigen (MacDonald & Nabholz 1986). Proliferative responses were detected early in the infestation, but they did not persist through the entire infestation; proliferative cells were not found at any time in the spleen. Putative immune effector cells in the draining lymph nodes may have migrated to the infestation site or were down-regulated as the infestation progressed. These data demonstrate that hosts have an immunologic component that is activated by louse antigens within a week of exposure to lice.

The systemic movement of proliferative cells or their products from the draining lymph nodes via the blood to the skin provides a mechanism for expression of generalized louse resistance. This does not preclude a role for the local conditions in the skin to contribute to resistance, because our studies show that second infestations on primary sites were smaller compared with naive sites. These data support the results of Bell et al. (1966, 1982) regarding the role of local and acquired immune components in the skin of resistant mice; however, they did not observe systemic resistance as we did. In their earliest attempts, new (naive) feeding sites were created by a second amputation and failed to express anamnestic resistance to lice (Bell et al. 1966). We felt that the second amputation of an adult animal following the primary infestation may have altered the lymphoid-adrenal axis with possible suppressive effects on resistance (Blalock & Smith 1985). Therefore, we used mice that were disabled at birth. In their second approach, naive and resistant skin were transplanted from resistant mice to athymic



Fig. 7. Skin biopsies taken 24 h after testing with soluble louse components and stained with hematoxylin-eosin. (Top) Naive animal, 250 $\times$ . (Bottom) Animal immunized with soluble louse components but not infested, 540 $\times$ . Notice the cellular infiltrate below the cartilage at the skin test site.

"nude" recipients. The skin from infestation sites maintained its resistant character, whereas naive skin failed to transfer louse resistance (Bell et al. 1982). However, their data can be interpreted consistently with our findings of systemic resistance. From our *in vitro* proliferation data, the inductive phases of resistance occur in the draining lymph nodes. Therefore, the lack of resistance in skin grafts from naive sites is expected because the antilouse

response begins in the draining lymph nodes rather than in the skin. Skin from infestation sites transfers resistance because it contains the infiltrating effector cells or products. The inability of athymic mice to develop resistance provides additional evidence that it is the T cells in draining lymph nodes which mediate louse resistance.

Immunization demonstrates that louse resistance can be induced in mice because undefined antigens in SLC significantly reduced primary louse burdens. Surprisingly, bovine serum albumin also lowered burdens, which can be explained by the cross-reactivity we observed between BSA and SLC in skin tests. Resistance induced by immunization appears to be the result of an immune-mediated skin response. This conclusion is supported by the histology of skin test sites from immunized mice, which was similar to the inflammatory histology described in infested skin (Nelson et al. 1972).

Our study, using the natural host of *P. serrata*, demonstrates an inducible systemic immune component in louse resistance mediated by a cutaneous inflammatory response at the feeding site. Furthermore, a simple immunization procedure sensitizes hosts with skin reactivity to louse antigens similar to those described during the infestation. The effectiveness of this immunization procedure encourages its application to a louse vaccine.

#### Acknowledgment

Our thanks go to D. Sonenshine for his helpful comments during manuscript preparation. This work was supported by USDA Cooperative Agreement 58-TB30-0-151 to S.K.W.

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Received for publication 8 February 1990; accepted 30 April 1990.