Comparative Medicine
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Vol 52, No 5
October 2002
Pages 429-432

Influence of Bedding Type on Mucosal Immune Responses

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The mucosal immune system interacts with the external environment. In the study reported here, we found that bedding materials can influence the intestinal immune responses of mice. We observed that mice housed on wood, compared with cotton bedding, had increased numbers of Peyer's patches (PP) visible under a dissecting microscope. In addition, culture of lymphoid organs revealed increased production of total and virus-specific IgA by PP and mesenteric lymph node (MLN) lymphocytes from mice housed on wood, compared with cotton bedding. However, bedding type did not influence serum virus-specific antibody responses. These observations indicate that bedding type influences the intestinal immune system and suggest that this issue should be considered by mucosal immunologists and personnel at animal care facilities.

For decades, wood chips have been the standard bedding material used at animal care facilities. Recently, however, some of these animal care facilities have switched to better defined bedding materials, such as cotton. Several concerns have been raised with use of wood chip bedding. Softwood bedding (such as pine chips) alters the activity of hepatic enzymes (1). Also, pine chips are more cytotoxic than is hardwood or grass materials (2). Finally, analysis has revealed significant lot-to-lot variability in the composition of wood chip bedding (2). These issues have led to changes in the bedding materials used at many animal care facilities.

We describe the ability of bedding type to influence intestinal immune responses. We undertook this study after observing that the size and number of small intestinal Peyer's patches (PP) were reduced in mice housed on cotton, compared with wood chip bedding. We found that mice housed on cotton bedding had lower intestinal humoral immune response after oral inoculation with an intestinal pathogen, rotavirus, than did mice housed on wood chip bedding.

Materials and Methods

Mice. Conventionally reared, 6- to 8-week-old BALB/cAnNTac female mice (Taconic Breeding Laboratories, Germantown, N.Y.) were housed in individual isolation units. Prior to rotavirus inoculation, serum from these mice did not contain rotavirus-specific antibodies, as determined by use of an enzyme-linked immunosorbent assay (ELISA).

Housing. On arrival at the animal care facility at the Wistar Institute (Philadelphia, Pa.), mice were separated into two groups and were housed on either irradiated iso-PAD cotton bedding (Harlan Teklad, Madison, Wis.) or Beta Chip bedding (Northeastern Products, Warrenburg, N.Y.), which is composed of any combination of three hardwoods: maple, birch, and beech. Five animals were housed in individual sterile microisolation cages. Bedding was changed weekly by trained animal care providers. Standard irradiated mouse feed (Pico-Vac Mouse Diet No. 5062, Purina Mills, Inc., St. Louis, Mo.) and autoclaved, acidified (pH range 2.5 to 3.5) water were available ad libitum. All manipulations were conducted in a class-II biosafety cabinet, using aseptic technique. This method provides cage-level protection, which facilitates maintenance of specific-pathogen-free conditions. Each year, serologic viral profiling of sentinel mice (representing approx.10% of the murine population) is performed by an independent diagnostic laboratory to ensure that the colony is free from minute virus of mice, murine parvovirus, pneumonia virus of mice, reovirus type 3, mouse hepatitis virus, Kilham's virus, Theiler's murine encephalomyelitis virus, Sendai virus, lymphocytic choriomeningitis virus, murine adenovirus, ectromelia and polyoma viruses, murine rotavirus, and *Mycoplasma pulmonis*. For the past five years, no screened mouse housed in the investigators' room within the animal care facility of the Wistar Institute has tested positive for any of these pathogens. In addition, the animal health profiles for all commercially purchased animals that are provided by individual vendors are reviewed weekly. All incoming mice from noncommercial sources are examined for pinworm ova (by tape test) and *Helicobacter* sp. (by use of polymerase chain reaction analysis of fecal pellets). The humane care and use of mice was ensured by approval of all research and animal care protocols by the Institutional Animal Care and Use Committees at the Children's Hospital of Philadelphia and the Wistar Institute.

Virus. Simian rotavirus, strain RRV, was originally obtained from N. Schmidt (Viral and Rickettsial Disease Laboratory, Berkeley, Calif.). The virus was grown in MA104 cells and was titrated for infectivity by use of plaque assay, as described (3).

Immunization of animals. Three weeks after arrival, adult mice were inoculated orally by proximal esophageal intubation with a dose of 5×10^6 plaque-forming units of RRV in a volume of 100 µl.

Harvest of tissues. Eight weeks after inoculation, lymphoid

Received: 4/02/02. Revision requested: 5/01/02 and 6/14/02. Accepted: 6/26/02.
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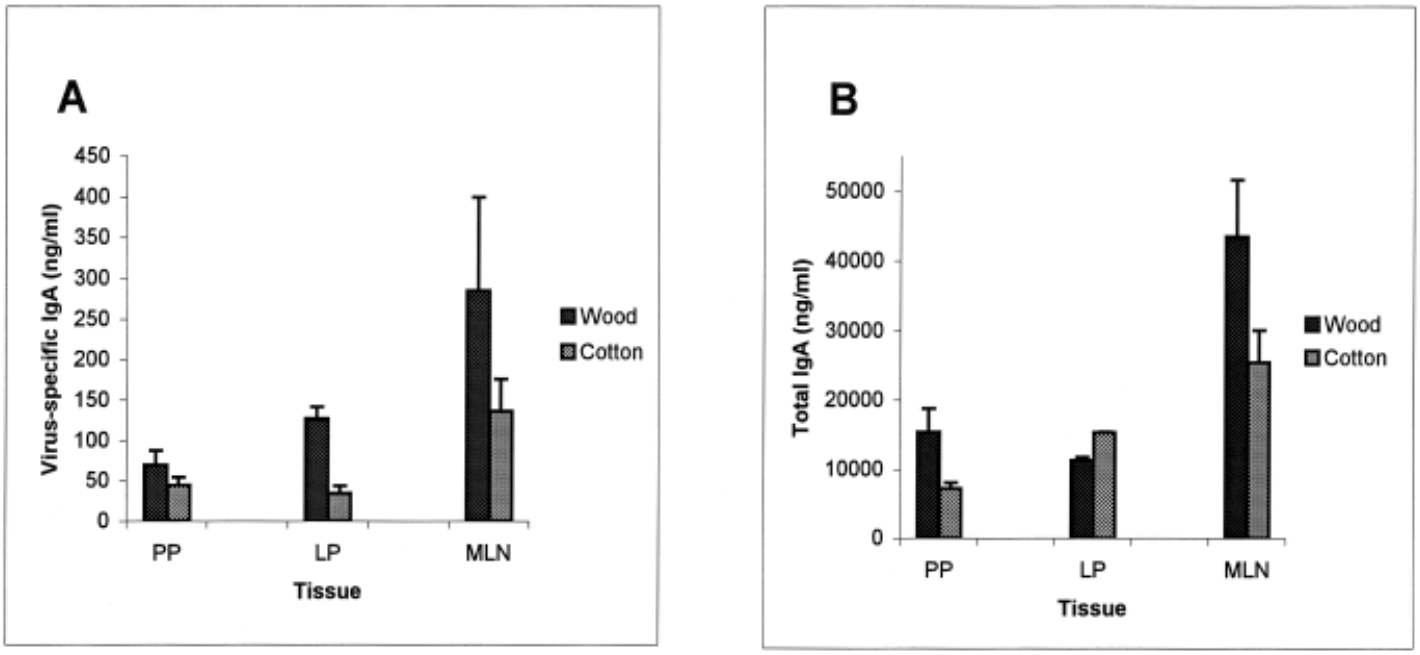

Figure 1. Production of virus-specific (A) and total (B) IgA after oral inoculation of adult BALB/c mice with rotavirus. On arrival at the animal care facility at the Wistar Institute, mice were housed on either wood chip or cotton bedding. Eight weeks after oral inoculation with 5×10^6 plaque-forming units (pfu) of rotavirus (strain RRV), mesenteric lymph nodes (MLN), Peyer's patches (PP), and lamina propria (LP) were harvested and cultured. Supernatants were subsequently tested for the presence of virus-specific and total immunoglobulins by use of an ELISA. Data depict mean concentrations of virus-specific or total IgA. Error bars indicate SEM.

cultures of lamina propria (LP), mesenteric lymph nodes (MLN), and PP were established, using five mice per group, as described (4). In brief, MLN and small intestines were isolated under sterile conditions. The MLN were washed twice in Iscove's modified Dulbecco's medium (GIBCO, Grand Island, N.Y.) containing gentamicin (50 µg/ml; JRH, Lenexa, Kans.) and were placed in individual wells of a 24-well plate (Becton Dickinson, Franklin Lakes, N.J.) with GALT medium (1 ml/well; Kennet's HY medium: 100 µg of streptomycin, 50 µg of gentamicin, and 0.25 µg of amphotericin B [Fungizone]/ml; all from JRH). After removal of PP, using a dissecting microscope at 30× magnification, the small intestine was opened longitudinally and cut into 5-cm segments. Small intestinal segments were washed twice in Hanks' balanced salt solution (HBSS; GIBCO) containing 50 µg of gentamicin/ml (JRH) and 25 m*M* HEPES (Mediatech, Washington, D.C.), once in HBSS with 0.05% EDTA to free the epithelial cells from the basement membrane and allow the intraepithelial lymphocytes to be removed, and twice in HBSS. Using the dissecting microscope, fat, mesentery, and connective tissues were removed from small intestinal segments. Eight 1 × 1-mm LP fragments from small intestine segments of each animal were removed and placed in individual wells of a 24-well plate containing 1 ml of GALT medium. The PP were washed three times and placed in individual wells of a 24-well plate containing 1 ml of GALT medium. Blood was drawn from mice by retro-orbital puncture, and sera were stored at 4°C.

Lymphoid organ cultures. The PP, MLN, and LP specimens were incubated for 5 days at 37°C in an atmosphere of 95% O_2 and 5% CO_2. Supernatant fluids were collected and subsequently tested for the presence of virus-specific and total immunoglobulins by use of an ELISA. Prior studies have indicated that the antibodies detected in lymphoid organ culture fluids are due to active production of antibodies by cultured tissues, not to passive transudation of serum-derived antibodies.

Detection of rotavirus-specific and total immunoglobulins by ELISA. The quantities of rotavirus-specific and total IgA and IgG in sera and supernatant fluids were determined by use of an ELISA as described (5).

Statistical methods. Differences in the concentrations of antibodies and numbers of PP were evaluated by use of the Mann-Whitney U test. Comparisons resulting in values of $P < 0.05$ were considered significant.

Results

Effect of wood chip bedding on mucosal virus-specific IgA and IgG responses in mice. To determine the effect of bedding type on mucosal humoral immune responses, we evaluated the production of rotavirus-specific IgA and IgG by intestinal tissues eight weeks after oral inoculation of mice with rotavirus. The B cells resident within PP, MLN, and LP in the intestinal tissues from mice housed on wood chip, compared with cotton bedding, produced more virus-specific IgA (Fig. 1A). In contrast, there was no difference in the quantities of virus-specific IgG produced by intestinal tissues from mice housed on wood, compared with cotton bedding (Fig. 2A).

Production of total intestinal IgA by PP and MLN lymphocytes. To determine the effect of bedding type on total intestinal IgA production, supernatant fluids from PP, MLN, and LP cultures were tested by ELISA eight weeks after oral inoculation of mice with rotavirus. The MLN and PP B cells from mice housed on wood chip, compared with cotton bedding, produced increased quantities of total IgA (Mann-Whitney U test, $P < 0.05$). However, production of total IgA or IgG by LP B cells was not influenced by bedding type (Fig. 1B and 2B).

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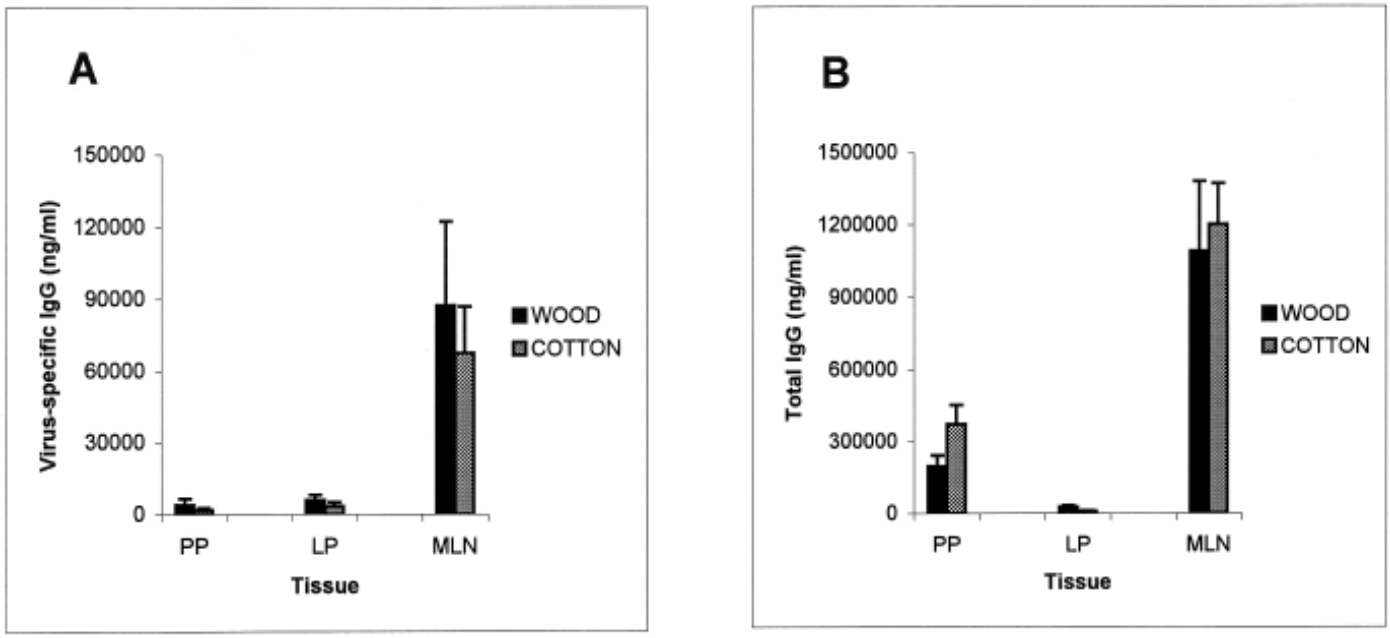

Figure 2. Production of virus-specific (A) and total (B) IgG after oral inoculation of adult BALB/c mice with rotavirus. On arrival at the animal care facility at the Wistar Institute, mice were housed on either wood chip or cotton bedding. Eight weeks after oral inoculation with 5×10^6 pfu of rotavirus (strain RRV), MLN, PP, and LP were harvested and cultured. Supernatants were subsequently tested for the presence of virus-specific and total immunoglobulins by use of an ELISA. Data depict mean concentrations of virus-specific or total IgG. Error bars indicate SEM.

Table 1. Quantities of small intestinal Peyer's patches visible in recently inoculated mice housed on wood chip, as compared to cotton, bedding*

	Bedding type	
	Wood chip	Cotton
Mouse 1	7	7
Mouse 2	11	5
Mouse 3	7	6
Mouse 4	7	8
Mouse 5	10	4
Mouse 6	8	8
Mouse 7	6	8
Mouse 8	10	8
Mouse 9	10	7
Mouse 10	10	8
Mean / Median	8.6 / 10	6.9 / 8

*5–6 wk old Balb/C mice were housed on wood chip or cotton bedding for 3 wks prior to oral inoculation with rotavirus. Eight wks after inoculation, the quantities of visible PP were determined by a blinded observer (SEC).

Numbers of PP observed in mice maintained on wood chip, compared with cotton bedding. To determine the capacity of bedding type to induce physiologic responses at the mucosal surface, a blinded observer (SEC) counted the numbers of small intestinal PP in 10 inoculated mice/group (Table 1). Significantly more PP were visible in mice maintained on wood, compared with cotton bedding (Mann-Whitney U test, 8.6 versus 6.4, $P < 0.05$).

Influence of bedding type on titer of serum rotavirus-specific IgA or IgG. Sera were obtained from mice eight weeks after oral inoculation with RRV and were tested for rotavirus-specific antibodies by use of an ELISA. There was no significant difference in the titer of serum virus-specific IgA or IgG responses between the mice housed on cotton, compared with those housed on wood chip bedding (data not shown).

Discussion

In this study, we found that bedding type influenced the nature of mucosal immune responses. We observed that wood chip, compared with cotton bedding, enhanced mucosal virus-specific IgA responses after primary inoculation of BALB/c mice with rotavirus. However, bedding type did not influence the titer of serum rotavirus-specific antibodies detected eight weeks after inoculation. In addition, we observed increased production of total intestinal IgA by PP and MLN B cells from mice housed on wood chip bedding. Finally, mice housed on wood chip bedding had more PP visible than did mice housed on cotton bedding.

Previous studies have indicated that bedding materials may influence various physiologic functions of laboratory animals. For example, pine chip bedding was found to inhibit oxidative microsomal enzymes in the rat liver in vivo (1). In addition, results of in vitro studies indicated that extracts from pine chip bedding were cytotoxic and that the degree of cytotoxicity varied significantly based on the source of bedding material (2). These observations suggest that bedding type may influence the outcome of drug metabolism and toxicity studies.

Little is known about the mechanisms by which bedding type may influence mucosal immune responses. However, previous studies indicated that germ-free mice, exposed to minimal antigenic stimulation, have greatly reduced intestinal lymphoid tissues and serum antibodies (6). In the study reported here, we observed that hardwood chip bedding induced hypertrophy of mucosal lymphoid structures, such as PP, and increased production of total IgA by PP and MLN lymphocytes from BALB/c mice. In addition, hardwood chip bedding appeared to act as an adjuvant, enhancing production of virus-specific IgA by intestinal lymphoid tissues after oral inoculation with rotavirus. Of interest, bedding type did not appear to influence serum antibody responses.

The mechanism by which hardwood chip bedding enhanced mucosal immune responses remains obscure. We hypothesize that hardwoods may contain a specific immunostimulatory com-

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pound. Other mucosal adjuvants, such as saponin, have been isolated from plants (7). An unrecognized immunostimulatory compound might enhance humoral immune responses by enhancing antigen-presenting cell function, altering the cytokine mileu in the lymphoid tissues of the small intestine, or increasing the state of cell activation. Alternatively, bedding type may influence the colonizing microflora of murine small intestine and, thus, affect mucosal immunity (8). Finally, investigators have reported that the immune responses of BALB/c mice are TH2-biased. Additional studies are needed to define the mechanisms and clinical relevance of the immunoenhancement induced by wood chip bedding.

We found that bedding type influenced the nature of mucosal immune responses in mice. These observations suggest that the choice of bedding type should be considered by mucosal immunologists, as well as investigators in drug metabolism and toxicity.

Acknowledgments

We thank Denise DiFrancesco and the animal caretakers at the Wistar Institute. We also thank the members of the Offit and Coffin laboratories for thoughtful discussions and technical assistance.

References

1. **Weichbrod, R. H., C. F. Cisar, J. G. Miller, R. C. Simmonds, A. P. Alvares, and T. H. Ueng.** 1988. Effects of cage beddings on microsomal oxidative enzymes in rat liver. Lab. Anim. Sci. **38:**296-298.
2. **Pelkonen, K. H. O. and O. O. P. Hanninen.** 1997. Cytotoxicity and biotransformation inducing activity of rodent beddings: a global survey using the Hepa-1 assay. Toxicology **122:**73-80.
3. **Offit, P. A. and R. D. Shaw**. 1983. Response of mice to rotavirus of bovine or primate origin as assessed by radioimmunoassay, radioimmunoprecipitation, and plaque reduction neutralization. Infect. Immun. **42:**293-300.
4. **Logan, A. C., K. N. Chow, A. George, P. D. Weinstein, and J. J. Cebra.** 1991. Use of Peyer's patch and lymph node cultures to compare local immune response to *Morganella morganii*. Infect. Immun. **59:**1024-1030.
5. **Khoury, C. A., K. Brown, J. Kim, and P. A. Offit.** 1994. Rotavirus-specific intestinal immune response in mice assessed by enzyme-linked immunospot assay and intestinal fragment culture. Clin. Diagn. Lab. Immunol. **1:**722-728.
6. **Olson, G. B. and B. S. Wostmann.** 1966. Lymphocytopoiesis, plasmacytopoiesis and cellular proliferation in nonantigenically stimulated germfree mice. J. Immunol. **97:**267-274.
7. **Elson, C. O. and M. T. Dertzbaugh.** 1999. Mucosal adjuvants, p.817-838. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology. Academic Press, Inc., San Diego, Calif.
8. **Savage, D. C.** 1999. Mucosal microbiota, p. 19-30. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal immunology. Academic Press, Inc., San Diego, Calif.

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