INFECTION AND IMMUNITY, Aug. 1997, p. 3267–3270 Vol. 65, No. 8
0019-9567/97/$04.00+0

The Baboon as a Nonhuman Primate Model for Assessing the Effects of Maternal Immunization with *Haemophilus influenzae* Type b Polysaccharide Vaccines

MICHAEL H. SHEARER,[1] ALEXANDER H. LUCAS,[2] PORTER W. ANDERSON,[3] KENNETH D. CAREY,[4] HAL B. JENSON,[5] TRAN C. CHANH,[6] JOHN R. STANLEY,[7] AND RONALD C. KENNEDY[1]*

Department of Microbiology and Immunology[1] and Department of Obstetrics and Gynecology,[7] University of Oklahoma Health Science Center, Oklahoma City, Oklahoma 73190; Children's Hospital Oakland Research Institute, Oakland, California 94609[2]; Department of Pediatrics, University of Rochester Medical Center, Rochester, New York 14642[3]; Department of Physiology and Medicine[4] and Department of Virology and Immunology,[6] Southwest Foundation for Biomedical Research, San Antonio, Texas 78207; and Departments of Pediatrics and Microbiology, University of Texas Health Science Center, San Antonio, Texas 78284[5]

Received 2 May 1997/Accepted 14 May 1997

These studies were performed to assess the utility of the baboon as a nonhuman primate model to evaluate vaccines for use in humans. Specifically, we examined the antibody response of baboons immunized during the third trimester of pregnancy with *Haemophilus influenzae* type b (Hib) polyribosylribitol phosphate (PRP) conjugate and unconjugated polysaccharide vaccines. Some of the vaccinated mothers failed to respond to a single immunization with unconjugated Hib PRP. Specific Hib PRP immunoglobulin G (IgG) but not IgM antibodies cross the baboon placenta and are detected in the offspring. Higher-titer baboon anti-Hib PRP did not express two previously defined cross-reactive human anti-Hib PRP idiotypes and was biased towards λ light-chain expression. Spectrotype analysis indicated that baboon anti-Hib PRP was restricted in heterogeneity and oligoclonal.

We are interested in utilizing the baboon as a nonhuman primate model for assessing the safety and immunogenicity of candidate vaccines in adults, pregnant females, and their infants, both full-term and premature. Our basis for selecting the baboon is because of similarities to humans in ontogeny, immunology (four IgG subclasses), reproductive physiology, placentation, and maternal-fetal transfer (4, 5, 8, 9, 15, 21, 22, 24). The advantages of the baboon over other commonly used, simian primates include the ease of timed pregnancies due to the estrogen-sensitive sex skin in cycling females, the comparative availability because baboons breed year round, the lack of susceptibility to herpes B virus, the relative ease of handling, and lower associated costs.

Immunization of the mother during the last trimester of pregnancy offers the theoretical advantage of providing the fetus with passively acquired maternal antibody prior to and after delivery. For many years maternal vaccination with tetanus toxoid during pregnancy has consistently demonstrated improved maternal and neonatal survival (3, 16, 18). Despite the success with tetanus toxoid vaccines, progress related to the study of maternal vaccination has been slow due to the lack of licensed vaccines for cases where maternally derived antibodies in the infant would be beneficial, as well as because of medical and legal issues (2). Organisms for which maternal vaccination may be advantageous to the infant include group B streptococcus, *Escherichia coli* K-12, meningococcus, and respiratory syncytial virus.

Perhaps the best studied bacterial vaccine in humans is that for *Haemophilus influenzae* type b (Hib) (25). These vaccines utilize the purified Hib capsular polysaccharide (PS), which consists of repeating units of polyribosylribitol phosphate (PRP). For Hib vaccines, the improved immunogenicity of glycoconjugates in which PRP was linked to a carrier protein was particularly relevant, especially among infants who responded poorly to PS antigens in general (25). Presently, Hib PRP is the only glycoconjugate vaccine licensed for use in infants. Studies utilizing Hib glycoconjugate vaccines have clearly demonstrated that systemic humoral immune responses are primarily responsible for protective immunity against Hib bacteremia and disease. Antibodies against PRP have been shown to be the primary component of serum bactericidal activity against the organism. For a comparative study in baboons, we selected Hib PRP vaccines as the model of choice for analyzing the effects of vaccination during pregnancy. Indeed, reports have described Hib glycoconjugate vaccination in humans during pregnancy (6).

MATERIALS AND METHODS

ELISA for detection of baboon antibodies to Hib PRP. Hib PRP conjugated to either human serum albumin or poly-L-lysine was adsorbed to the solid phase (7). A 1:100 dilution of baboon serum was added to the Hib PRP-coated wells, and immunoglobulin M (IgM) and IgG specific reactivities were detected with cross-reactive monoclonal anti-human IgM and IgG class-specific reagents (Fisher Biotech, Pittsburgh Pa.). Specific details of the enzyme-linked immunosorbent assay (ELISA) are described elsewhere (1). Positive-reactivity cutoff values were determined as optical densities at 405 nm of greater than 0.1 and 0.08 for IgM and IgG, respectively. The cutoff values represent three times the mean optical densities at 405 nm obtained with a panel of Hib PRP-negative sera (antibody concentration of less than 50 ng/ml as determined by radioantigen binding assay) at a 1:50 dilution and have been described for other assay systems elsewhere (23).

ELISA for detection of anti-Hib PRP L-chain isotype levels. κ and λ anti-Hib PRP antibody concentrations were measured by an ELISA similar to that described previously (7). In brief, 96-well microtiter plates were coated with Hib PRP coupled to poly-L-lysine. Dilutions of test sera were incubated with an equal

* Corresponding author. Mailing address: Department of Microbiology and Immunology, College of Medicine, University of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73109. Phone: (405) 271-5630. Fax: (405) 271-6339.

TABLE 1. Antibody responses to Hib unconjugated PS vaccination in pregnant baboons and their offspring

Baboon	Day post-vaccination	Total antibody to Hib PRP (ng/ml)	Anti-Hib PRP	
			IgM	IgG
Mother 5	0	795	+	−
	20	1,185	+	−
Offspring 5		<50	−	−
Mother 6	0	540	+	+
	20	1,930	+	+
Offspring 6		52	−	+
Mother 7	0	1,405	+	+
	30	1,510	+	+
Offspring 7		300	−	+
Mother 8	0	320	+	−
	40	350	+	−
Offspring 8		<50	−	−

volume of buffer alone or buffer that contained 10 μg of Hib PRP per ml. After washing, κ antibodies were detected with alkaline phosphatase-coupled goat antibodies specific for human κ chains, and λ antibodies were detected with alkaline phosphatase-coupled goat antibodies specific for human λ (Tago, Burlingame, Calif.). These antibodies demonstrated cross-reactivity with the baboon light (L)-chain analogs. We have previously characterized serologic cross-reactions among human and baboon immunoglobulin preparations (19, 20). The reactivities of anti-human heavy- and L-chain reagents with baboon heavy and L chains have been described previously (1, 10). For quantitation of antibodies, the absorbance values obtained in the wells incubated with test sera diluted in buffer that contained soluble Hib PRP were subtracted from the absorbance values in the respective wells incubated with test sera diluted in buffer alone. Purified PRP-specific human monoclonal antibodies served as standards for quantification (data not shown).

Radioantigen binding assay for detection of total antibody to Hib PRP. Total antibody to Hib PRP was measured by radioantigen binding assays with ^{125}I-labeled Hib PRP, as previously described (10, 11). Antibody concentrations were determined from a standard curve generated by using dilutions of a serum reference pool, obtained from the Center for Biological Evaluation and Research, that contained 70 μg of Hib PRP antibody per ml. The sensitivity of this assay was 50 ng/ml.

Isoelectric focusing (IEF) and Western blotting. Serum samples (5 μl) were focused in 0.8-mm polyacrylamide gels, as previously described (10, 11). The focused samples were electrically blotted onto nitrocellulose, and PRP-specific antibodies were detected by reaction with ^{125}I-Hib PRP and subsequent exposure to X-ray film.

Immunization of baboons. Baboons with known conception dates were vaccinated intramuscularly 20 to 40 days prior to delivery with either 5 μg of Hib PRP unconjugated vaccine (supplied by the National Institutes of Health) or 10 μg of the Hib PRP conjugate vaccine (HbOC; Wyeth-Lederle Pediatric Vaccines) in saline. Serum was obtained from the mothers prior to vaccination and delivery and from the infants within 24 h of birth. To evaluate antibody decay in Hib conjugate-vaccinated mothers, serum was obtained approximately 1 and 3 months postpartum. To examine the amnestic response and the effect of adjuvant and to induce high-titer responses to characterize the antibody, three conjugate-vaccinated females were reinjected after 14 months with Hib conjugate vaccine (HbOC) emulsified in Freund's incomplete adjuvant. They received three monthly injections. Baboons had not received the diphtheria-pertussis-tetanus toxoid combination vaccine prior to initiation of this study.

RESULTS

Examination of Hib PRP unconjugated vaccines. Only one of the four mothers vaccinated with the Hib PRP unconjugated vaccine demonstrated a greater-than-threefold increase in anti-Hib PRP titers (Table 1). In this mother (no. 6), IgG anti-Hib PRP was detected prior to birth and was transplacentally transferred to her infant. A similar situation was observed in a second mother who appeared to transfer IgG anti-Hib PRP to her infant. For the two mothers (no. 5 and 8) in which no IgG anti-Hib PRP was detected, no anti-Hib response was observed in the offspring. This data suggests that IgG and not IgM anti-Hib PRP is transferred to the baboon infants.

Examination of Hib PRP conjugate vaccines. Each of the three conjugate-vaccinated mothers had Hib PRP titers increase by greater than threefold (Table 2). One mother (no. 9) responded with lower anti-Hib PRP levels as the result of glycoconjugate vaccination when compared to the other two mothers (increases in titers from 46- to 53-fold). In each of the three mothers that received the Hib PRP vaccine, an anti-Hib PRP IgG response was observed following vaccination. The decay of the anti-Hib PRP response in conjugate-vaccinated mothers over a period of approximately 4 months was greatest in the mothers that exhibited the best antibody response at approximately 30 days after vaccination. Anti-Hib PRP titers decreased in mothers 10 and 11 by approximately three- and twofold, respectively, over a 100-day period. A specific antibody response to Hib PRP can cross the baboon placenta and be detected in the offspring. IgG but not IgM antibodies to Hib PRP cross the baboon placenta and are detected in the infant. The anti-Hib PRP titers that were passively acquired by the infant were not reflective of the anti-Hib PRP titers present in the mother at the time of birth.

Characterization of high-titer antibody response to Hib PRP after booster vaccination. Baboons that had previously received a single injection of the HbOC vaccine in saline were rested for a 14-month period and then boosted three times with the HbOC conjugate vaccine in Freund's incomplete adjuvant. These baboons produced high titers of anti-Hib PRP antibody as determined by the Hib PRP radioantigen binding assay. Following each vaccination, a boost in the anti-Hib PRP titers was observed (Table 3). Further serologic characterization indicated that baboons immunized with the HbOC conjugate vaccine failed to express the two previously characterized cross-reactive human anti-Hib PRP idiotypes (Ids), HibId-1 and HibId-2 (data not shown). This lack of human anti-Hib PRP Id expression is not the result of low titers of anti-Hib PRP induced in the baboon. It is possible that the use of adjuvants in the HbOC booster vaccinations may have altered the Id expression, and hence the human anti-Hib PRP Ids were not detected. Unlike the human anti-Hib PRP response, the anti-Hib PRP responses in the baboons appear to be biased towards λ L-chain expression. Like those of humans, baboon

TABLE 2. Antibody responses to Hib conjugate PS vaccination in pregnant baboons and their offspring

Baboon	Day post-vaccination	Total antibody to Hib PRP (ng/ml)	Anti-Hib PRP	
			IgM	IgG
Mother 9	0	220	+	−
	36	700	+	+
	65	640	+	+
	105	490	+	+
Offspring 9		52	−	+
Mother 10	0	230	+	−
	39	12,500	+	+
	65	5,700	+	+
	105	4,150	+/−	+
Offspring 10		1,174	−	+
Mother 11	0	80	+	−
	34	3,700	+	+
	64	2,100	+	+
	104	1,850	+/−	+
Offspring 11		2,050	−	+

TABLE 3. Characteristics of anti-PRP antibody responses in baboons vaccinated with HbOC

Baboon	Serum anti-PRP (μg/ml)[a]					λ/κ[b]
	Prevaccination	14 days after:			28 day after third booster	
		First booster	Second booster	Third booster		
4272	0.4	23.0	54.0	143.0	139.0	3.1
6717	1.6	149.0	788.0	2,202.0	1,943.0	6.0
3548	1.7	62.0	568.0	4,549.0	1,635.0	2.9

[a] Levels of anti-PRP were determined by a radioantigen binding assay. Values were calculated from a standard curve generated with a standard reference antiserum containing 70 μg of anti-PRP/ml. The values shown are the arithmetic means from at least three separate determinations. Sera were obtained 14 or 28 days after the first, second, or third booster injection.

[b] Ratio of λanti-PRP to κ anti-PRP (both in micrograms per milliliter) in sera obtained 28 days after the third booster vaccination.

anti-Hib PRP responses were oligoclonal and of limited complexity, as only a few clonotypes were observed by IEF. These baboon anti-PRP spectrotypes exhibited restricted pH ranges, from 7.4 to 7.8, and similar clonotypes were observed among the three different baboon anti-PRP preparations (Fig. 1). Anti-PRP spectrotypes unique to an individual baboon were also observed and exhibited more-acidic pH ranges.

DISCUSSION

The overall goal of this study was to examine the baboon as a nonhuman primate model to evaluate vaccination during pregnancy. Specifically, we chose to examine the antibody responses of pregnant baboons immunized with Hib PRP conjugate and unconjugated PS vaccines that have been used previously to vaccinate humans. Not all pregnant baboons appear to respond to a single immunization with Hib PRP unconjugated PS vaccine during the third trimester (response defined by a threefold increase in anti-PRP levels). Since the unconjugated PS-vaccinated baboons exhibited preexisting anti-Hib PRP, this may have affected the ability of the baboons to respond to a single injection. Preexisting anti-Hib PRP has been observed in adult humans undergoing Hib PRP vaccination (17). Specific antibodies to Hib PRP can cross the baboon placenta and be detected in the offspring. IgG but not IgM antibodies to Hib PRP cross the baboon placenta. These results clearly parallel the situation for Hib in the human population, except that the titers of Hib-specific antibodies following a single injection in saline in the vaccine-responding animals appeared to be lower. Thus, the baboon may be a less sensitive indicator of antibody responses induced following glycoconjugate vaccination compared to humans. The presence of preexisting antibodies to Hib PRP may also have affected the response to the Hib PRP conjugate vaccine, similar to that reported for humans.

In order to raise high-titer anti-PRP antibodies and characterize these humoral immune responses, we reimmunized conjugate-vaccinated baboons three times with the Praxis-Lederle Hib conjugate (HbOC) vaccine in Freund's incomplete adjuvant. Our purpose in generating high-titer anti-Hib PRP in baboons was twofold. First, we wanted to compare baboon anti-Hib PRP Id expression to that of humans. Second, we wanted to provide large quantities of high-titer baboon anti-Hib PRP for further serologic characterization. Following each vaccination, a significant boost in the anti-Hib PRP titers was observed. Following glycoconjugate vaccination, the baboon is capable of producing high-titer antibody responses. In a previous study, anti-Hib PRP responses induced in nonhuman primates, including baboons, exhibited similarities to those in humans, both immunochemically and in protection experiments within in vivo to models of Hib bacteremia (10).

Further serologic characterization indicated that baboons immunized with the HbOC conjugate vaccine failed to express the two previously defined cross-reactive human anti-Hib PRP Ids, HibId-1 and HibId-2 (12, 13). The baboon and human anti-Hib PRP responses appear to differ in Id V_L region expression. An additional difference between the human and baboon anti-Hib PRP responses is that the baboon antibodies are biased towards λ L-chain expression, whereas human anti-Hib PRP responses favor the expression of κ L chains. In part, this observation may explain the lack of expression of HibId-1 in the baboon anti-Hib PRP response, since HibId-1 is associated with a human V-region κ-chain germ line gene family, designated A2. Similar to those of humans, baboon anti-Hib PRP responses were oligoclonal and of limited complexity, as only a few clonotypes were observed by IEF and spectrotype analysis.

Other examples of nonhuman primates that are closely related to humans but may not be appropriate models for maternal vaccination studies are the macaca species and the chimpanzee. Macaques are not suitable for these studies primarily because of significant differences in humoral immunity (three IgG subclasses) and placentation (14). Humans, like baboons, are single discoid, while macaques are double discoid. This difference in placentation raises concerns about the equivalence of maternal-fetal transfer of immunoglobulins. Macaca species can also carry herpes B virus, which can cause fatal neurologic infections in exposed animals and among animal care workers and handlers. Also, macaques breed seasonally. Although chimpanzees are most closely related to humans, the expense of developing the chimpanzee model would be prohibitive. Also, the chimpanzee is an endangered species.

Our data suggests that the baboon may represent a useful nonhuman primate model to examine the effects of vaccination

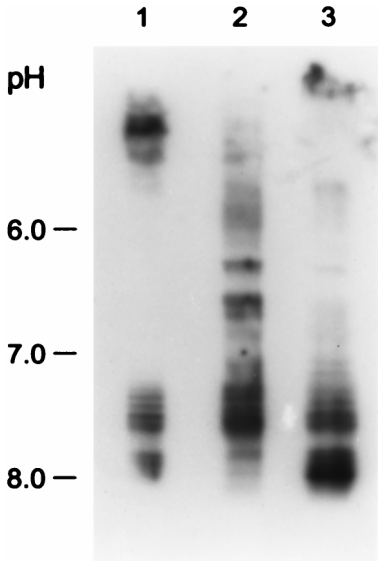

FIG. 1. Autoradiogram of IEF Western blot reacted with ^{125}I-PRP. Results for sera from baboons 3548 (lane 1), 6717 (lane 2), and 4272 (lane 3), taken 218 days after the third vaccination with HbOC, are shown. Clonotypes shared among the three baboon anti-PRP preparations are observed at pH 7.4 to 7.8. Clonotypes unique to the individual baboons are observed at more-acidic pH ranges.

during pregnancy. The baboon Hib response demonstrates not only a number of close similarities but also distinct structure and functional characteristics when compared to human antibodies induced by Hib glycoconjugate vaccination.

ACKNOWLEDGMENT

This work is supported in part by contract NOI-AI-35156 from the National Institutes of Health.

REFERENCES

1. **Attanasio, R., J. S. Allan, S. A. Anderson, T. C. Chanh, and R. C. Kennedy.** 1991. Anti-idiotypic antibody response to monoclonal anti-CD4 preparations in nonhuman primate species. J. Immunol. **146:**507–514.
2. **Baker, C. J.** 1990. Immunization to prevent group B streptococcal disease: victories and vexations. J. Infect. Dis. **161:**917–921.
3. **Chen, S. T., G. Edsall, M. M. Peel, and T. A. Sinnathuray.** 1983. Timing of anti-neonatal tetanus immunization for effective protection of the neonate. Bull. W. H. O. **61:**159–165.
4. **Damian, R. T., N. D. Greene, and S. S. Kalter.** 1971. IgG subclasses in the baboon *Papio cynocephalus*. J. Immunol. **106:**246–257.
5. **Del Portillo, H. A., G. W. Schmidt, and R. T. Damian.** 1987. Immunochemical analysis of baboon *Papio cynocephalus* IgG subclass. Vet. Immunol. Immunopathol. **16:**201–214.
6. **Englund, J. A., W. P. Glezen, C. Turner, J. Harvey, C. Thompson, and G. R. Siber.** 1995. Transplacental antibody transfer following maternal immunization with polysaccharide and conjugate *Haemophilus influenzae* type b vaccines. J. Infect. Dis. **171:**99–105.
7. **Granoff, D. M., P. G. Shackleford, J. P. Pandey, and E. G. Boies.** 1986. Antibody responses to *Haemophilus influenzae* type b polysaccharide vaccine in relation to Km(1) and G2m(23) immunoglobulin allotypes. J. Infect. Dis. **154:**257–264.
8. **Hankins, G.** 1990. Transplacental transfer of zidoviridine (AZT) in near term pregnant baboons. Am. J. Obstet. Gynecol. **163:**728–732.
9. **Hendricx, A. G., and D. C. Kramer.** 1971. Embryology of the baboon, p. 3–30. University of Chicago Press, Chicago, Ill.
10. **Kim, K. H., M. K. Park, C. Peeters, M. H. Shearer, R. C. Kennedy, and M. H. Nahm.** 1994. Comparison of nonhuman primate antibodies against *Haemophilus influenzae* type B polysaccharide with human antibodies in oligoclonality and in vivo protective potency. Infect. Immun. **62:**2426–2431.
11. **Lucas, A. H.** 1988. Expression of crossreactive idiotypes by human antibodies specific for the capsular polysaccharide of *Haemophilus influenzae* b. J. Clin. Invest. **81:**480–486.
12. **Lucas, A. H., and D. M. Granoff.** 1990. A major crossreactive idiotype associated with human antibodies to the *Haemophilus influenzae* b polysaccharide. Expression in relation to age and immunoglobulin G subclass. J. Clin. Invest. **85:**1158–1166.
13. **Lucas, A. H., F. H. Azmi, C. M. Mink, and D. M. Granoff.** 1993. Age-dependent V region expression in the human antibody response to the *Haemophilus influenzae* type b polysaccharide. J. Immunol. **150:**2056–2061.
14. **Martin, L. N.** 1982. Chromatographic fractionation of rhesus monkey *Macacca mulatta* IgG subclasses using DEAE cellulose and protein A-sepharose. J. Immunol. Methods **50:**319–329.
15. **Pauerstein, C. J., C. A. Eddy, H. D. Croxatto, R. Hess, T. M. Siler-Khodr, and H. B. Croxatto.** 1978. Temporal relationships of estrogen, protesterone, and luteinizing hormone levels to ovulation in women and infrahuman primates. Am. J. Obstet. Gynecol. **130:**876–886.
16. **Rahman, M., L. C. Chen, J. Chakraborty, M. D. Yunus, A. I. Chowdhury, A. M. Sarder, S. Bhatia, and G. T. Curlin.** 1982. Use of tetanus toxoid for the prevention of neonatal tetanus. Bull. W. H. O., **60:**261–267.
17. **Robbins, J. B., J. C. Parker, Jr., R. Schneerson, and J. K. Whisnant.** 1973. Quantitative measurement of natural and immunization-induced *Haemphilus influenzae* type b capsular polysaccharide antibodies. Pediatr. Res. **7:**103–110.
18. **Schofield, F. D., V. M. Tucker, and G. R. Westbrook.** 1961. Neonatal tetanus in New Guinea: effect of active immunization in pregnancy. Br. Med. J. **2:**785–789.
19. **Shearer, M. H., H. B. Jenson, K. D. Carey, T. C. Chanh, and R. C. Kenney.** 1994. Production and characterization of murine monoclonal antibodies specific for baboon IgG heavy and light chains. J. Med. Primatol. **23:**382–387.
20. **Shearer, M. H., F. L. Stevens, H. B. Jenson, T. C. Chanh, K. D. Carey, G. L. White, and R. C. Kennedy.** 1995. Serologic cross-reactions among primate immunoglobulins. Dev. Comp. Immunol. **19:**547–557.
21. **Stevens, V. C., S. J. Sparks, and J. E. Powell.** 1971. Levels of estrogens, pregestogens, and luteinizing hormone during the menstrual cycle of the baboon. Endocrinology **87:**658–666.
22. **Stevens, V. C.** 1978. Fertility regulation with humoral antigens: models for evaluating immunologic methods p. 284–299. *In* N. J. Alexander (ed.), Symposium on animal modes for research in contraception and fertility. Harper and Row, Hagerstown, Md.
23. **Wolf, H., R. Q. Warren, G. W. Stunz, K. R. Shuler, P. Kanda, and R. C. Kennedy.** 1992. Fine specificity of the murine antibody response to HIV-1 gp160 determined by synthetic peptides which define selected epitopes. Mol. Immunol. **29:**989–998.
24. **Yamamoto, Y., A. T. Manyon, Y. Osawa, R. Y. Kirdani, and A. A. J. Sandberg.** 1978. Androgen metabolism in the baboon: a comparison with the human. Steroid Biochem. **7:**751–759.
25. **Zangwill, K. M., J. D. Wenger, R. W. Sutter, and S. C. Hadler.** 1993. Recommendations for use of *Haemophilus* b conjugate vaccines and a combined diphtheria, tetanus, pertussis, and *Haemophilus* b vaccine. Recommendations of the Advisory Committee on Immunization Practices (ACIP). Morbid. Mortal. Weekly Rep. **42:**1–15.

Editor: V. A. Fischetti