Evaluation of the Humoral Immune Response of Children with Low Level Lead Exposure

J. Routt Reigart[1] and Charles D. Graber[2]

[1]*Assistant Professor, Department of Pediatrics*
Medical University of South Carolina, Charleston, S.C.

[2]*Professor, Department of Microbiology*
Medical University of South Carolina, Charleston, S.C.

Chronic and subacute exposure to lead in preschool children has well documented effects on the hematopoetic, neurological, renal, and cardiovascular systems. Reports in animal models have suggested that exposure to lead may also interfere with patterns of the immune response. Several reports have centered on inhibition of antibody response (KIRYACHKO, 1957, GIULIANI, et al, 1955, BELLI, 1955), hypocomplementemia (KIRYACHKO, 1957), and deficient response to viral (GAINER, 1974) and bacterial (HEMPHILL, et al, 1971) infections in animals. To date, however, there have been no reports on the effects of low level chronic lead exposure on the immune systems of children. In order to investigate the immune response in children exposed to abnormal amounts of lead, the following study was undertaken. Examined were levels of complement and immunoglobulins in these children, as well as the anamnestic response to a specific soluble antigen.

MATERIALS AND METHODS

Nineteen black children in a preschool age group (4-6) were the volunteer subjects of the study. Careful informed consent was obtained in all cases. Twelve of these children had evidence of increased lead absorption and metabolic impairment based on confirmed blood lead values $\geq$ 40 µgm/dl by atomic absorption spectroscopy and elevated levels of free erythrocyte porphyrins (FEP).

Blood leads were performed in duplicate by the modified Delves cup method (BARTHEL, 1972) on a Perkins-Elmer Model 303 Atomic Absorption Spectrophotometer. Free erythrocyte porphyrins were determined by Chisolm's (CHISOLM, et al, 1974) method with acidified acetone extraction on the Space Sciences Model 201 photofluorimeter. For this method, a porphyrin level $>$ 7.27 µgm/dl for children with hematocrit $>$ 35 is considered indicative of increased lead absorption with metabolic effect.

Serum samples were obtained prior to and eight days after immunization with 0.5 ml of tetanus toxoid. Since all of the children had received their initial primary series and a single booster of DPT at least 52 weeks prior to this immunization, the tetanus toxoid served as a recall antigen and promoted an anamnestic response. At the second bleeding, all children received diptheria and pertussis antigens to complete their preschool immunizations. Ten ml of blood was obtained at each bleeding

and the serum was used to perform the major immune globulin (IgG, IgM, IgA) determinations using the radial immune diffusion technique (Hyland Laboratories, Costa Mesa, California). Assay of the third component of complement (C_3) was also run on pre and post-immunization sera employing the same method. CH_{100} (total complement levels) were also run on the paired sera using immune radial hemolysis. (Kalistad Laboratories, Minneapolis, Minnesota).

Titration of tetanus antitoxin in the sera of 7 of 12 test children and 6 of the 7 controls was done in Swiss-Webster mice using the Massachusetts Public Health Biologic Laboratories method. A secondary response titration L+/100 level, which anticipates a serum titre in the subject in the region of .025 to 50 antitoxin units per ml, was used (L+/100 level is a dose of tetanus toxin which mixed with a L+/100 antitoxin unit will allow survival of the mouse with symptoms of tetanus 7 days after injection). Symptoms observed in such animals were stiffening of the leg, opisthotonos, and inability to close the jaw. Normal protective tetanus antitoxin level in children 4-6 years has been calculated at .01 antitoxin unit.

RESULTS

The twelve children in the experimental group had blood lead values in the range 41-51 µgm/dl (mean 45.3 µgm/dl) and FEP in the range of 7.7-12.7 (mean 10.4 µgm/dl). The children in the control group had blood lead levels $\leq$ 30 µgm/dl (range 14-30 µgm/dl, mean 22.6) and FEP $<$ 7.27 µgm/dl (range 4.1-5.9 µgm/dl, mean 5.0).

Table I shows only one of 12 lead burdened children to have a low immunoglobulin level prior to the booster injection of tetanus toxoid. This child's IgM level was 36 mg/dl and became a normal 67 mg/dl after toxoid injection. Two lead burdened children had a high IgG level (1700, 1700 mg/dl) prior to immunization, but one child returned to normal limits (1200 mg/dl) while the other became somewhat higher (1900 mg/dl).

Only one of the children with normal lead levels initially was hypogammaglobulinemic (IgG, 600 mg/dl). This child reached normoglobulinemia following toxoid immunization (IgG, 640 mg/dl). Three of the children with normal lead levels had somewhat elevated IgG levels (1500, 2100, 1550 mg/dl). Following toxoid immunization, two values remained the same (1500, 2100 mg/dl), but the other dropped to a normal level (1250 mg/dl). Two normals had somewhat elevated IgM values (250-300 mg/dl), but following toxoid injection, both fell to a level of 138 mg/dl.

Total complement as measured by immune hemolysis diffusion showed 5 of 12 lead burdened children to have markedly low levels (10, 10, 3.1, 10, 15) prior to booster immunization. Two of these children reached normal levels following injection of toxoid. All seven normal children had low total complement (10, 15.7, 14, 19.5, 20, 12.5, 19). One child became normocomplementemic after immunization.

TABLE 1

IMMUNOGLOBULIN, COMPLEMENT, AND TETANUS ANTITOXIN LEVELS IN LEAD BURDENED AND NORMAL CHILDREN (4-6 YEARS) PRIOR TO AND AFTER IMMUNIZATION WITH TETANUS TOXOID

Children		Ig Levels*				Complement Levels**						Tetanus Antitoxin Levels		
		IgG (640-1420)		IgA (52-120)		IgM (40-180)		CH-100 (25-50)		C_3 (123-167)				
		BTI	PTI	BTI	PTI	BTI	PTI	BTI	PTI	BTI	PTI			
NORMAL (≤30 mcg/dl lead) 7	LO	1	0	0	0	0	0	7	6	3	3	NL 6	BTI# 6 ≥.32 AU/ml	PTI@ 5 >50 AU/ml
	NL	3	5	7	7	5	7	0	1	4	4			
	HI	3	2	0	0	2	0	0	0	0	0			(25-50 AU/ml)
LEAD BURDENED (≥40 mcg/dl lead) 12	LO	0	0	0	0	1	0	5	3	7	7	LEAD 7	7 ≥.32 AU/ml	3 >50 AU/ml
	NL	10	11	12	12	11	12	7	9	4	5			
	HI	2	1	0	0	0	0	0	0	1	0			4 (25-50)

* Immune deficiency measurement

** CH-100- measured by immune radial hemolysis, C_3 by radial dysfusion

BTI = before tetanus immunization - DPT given to all children at least 52 weeks prior
.01 AU= protective level

@ PTI = post tetanus immunization
10-20 AU= normal response

C_3 levels were low in 7 of 12 lead burdened children (115, 120, 110, 122, 120, 105, 57 mg/dl). After tetanus toxoid booster, the serum C_3 level in two children became normocomplementemic (105-148, 110-125 mg/dl) while this component dropped in two other children from a normal to somewhat lower level (120-105, 122-110 mg/dl). Three of the seven normal children had reduced C_3 component in their serum (90, 120, 115 mg/dl) and this level remained generally the same in these children with immunization (101, 105, 120 mg/dl).

Four children in this group had normocomplementemic levels prior to and following immunization.

Seven lead burdened children's serum used to protect mice against infection of tetanus toxoid demonstrated levels in excess of .32 antitoxin units (A.U.) per milliliter (.01 A.U. level is protective for a 4-6 age child). These levels rose to greater than 50 A.U. in three children (normal level for 4-6 year child following booster tetanus is 10-20 A.U.) and to a level between 25-50 A.U. in four children with excess lead burden. Six children with normal lead levels all had tetanus antitoxin levels in excess of .32 A.U./ml prior to immunization. Five of these children following the injection of tetanus toxoid gave levels in excess of 50 A.U./ml while the serum of one child showed a level between 25-50 A.U.

DISCUSSION

The limited study described above indicates that low level lead toxicity as documented by FEP evaluation and abnormal blood lead in the lead burdened children had no significant effect on the major immunoglobulins, total complement, C_3, or the anamnestic response to tetanus toxoid.

Although a tendency to low total complement levels existed in both groups, no significantly lower levels of either CH_{100} or C_3 was evident in the lead children before or after toxoid immunization. These findings differ somewhat from those of Miyazaki (MIYAZAKI, 1959) who found guinea pig serum complement activity inhibited *in vitro* by lead and Kiryachko (KIRYACHKO, 1957) who demonstrated that lead poisoning in rabbits reduced total serum complement. The major immunoglobulins were not significantly different in either study group prior to and post-immunization. This was particularly true of IgG and suggests that regardless of immunogen chosen, difficulty in detecting immunohypogammaglobulinemia would occur.

The tetanus toxoid immunogen was chosen as an antigen that was a necessary part of the subject's routine immunization series and, therefore, not a cause for ethical concern. In addition, it was expected to produce a relatively easily measured anamnestic response at the L+/100 level. Indeed, the response in most children was so high ($>$ 50 antitoxic units) that it was difficult to precisely delimit the antitoxin levels to tetanus.

Whether our results would have been the same in our children using a particulate instead of a soluble antigen as reported by Giuliani, et al (GIULIANI, et al, 1955, BELLI, 1955) for lead poisoned animals is uncertain. These authors used streptococcal and brucella vaccines which promote IgM production initially followed by IgG. Both vaccines were given as primary immunogen and it was found that lead interfered with primary antibody production. Our immunogen, tetanus toxoid, produced neutralizing antitoxin (IgG) while concomitantly some non-protective hemagglutinating antibody of the IgM type was produced. The IgG normoglobulinemia in our children, pre-immunization, and the prolific response to the soluble toxoid antigen suggests that neither group of children would have experienced depression of the primary antibody response to a particulate antigen.

It is clear that low level lead toxicity, as documented in these children by FEP elevation and abnormal blood leads, had no significant effect on complement levels, total immunoglobulin levels, or the response to the antigenic stimulus of a tetanus booster. It is possible that these same children may have deficient response to a primary antigenic stimulus, but we consider this unlikely in light of their ability to maintain adequate IgG levels, presumably in response to a variety of antigenic stimuli. It remains to be determined whether there are specific defects in response to other antigens such as typhoid. In addition, it would probably be useful to examine the immunological response of children with more severe lead toxicity to determine whether impairment of immune response may be a dose-related phenomenon.

SUMMARY

Twelve lead-exposed children, with evidence of metabolic impairment, and seven non-lead exposed children were examined for evidence of impairment of their immunological response. There were no differences between the control group and the lead exposed group with reference to complement levels, immunoglobulins, or anamnestic response to the tetanus toxoid antigen. It remains to be demonstrated whether or not there is deficient response to primary immunization, whether other antigens are more affected by lead, or whether impairment of humoral immune response requires a more serious degree of lead intoxication.

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