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Mucocutaneous lymph node syndrome
polyarteritis nodosa
serum immunoglobulin E

Elevated Levels of Immunoglobulin E in the Acute Febrile Mucocutaneous Lymph Node Syndrome

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Extract

Mucocutaneous lymph node syndrome (MCLS) is a newly recognized disease characterized by fever persisting for more than 5 days, an erythematous skin eruption, conjunctival congestion, dry red fissured lips, reddened tongue, palms, and soles, nonpurulent lymphadenopathy, and sometimes diarrhea, arthralgia, and aseptic meningitis. Additional features may include carditis, pericarditis, aneurysmal dilation and thrombosis of coronary arteries, and sudden death. There is a striking similarity of fatal cases to infantile polyarteritis nodosa, a disease recently reported to be associated with elevated levels of serum IgE. Indeed, it is likely that MCLS represents a disease which can progress to polyarteritis nodosa in infants and young children.

The paired acute and convalescent serum IgE levels of 20 subjects with acute nonfatal MCLS were studied along with 20 near-age unaffected controls from the same communities in Japan. The results indicate that most if not all subjects with MCLS in the study had an elevation of total serum IgE during the acute phase of the disease (geometric mean 157 IU/ml compared with the control value of 38 IU/ml, $P = 0.005$). The level appeared to reach a peak 1–2 weeks after onset and declined over the ensuing 1–2 months.

Speculation

It may be that only subjects who are genetically programmed for a brisk IgE response to either a rickettsial-like agent or to some other unidentified immunogen will develop the usual manifestations of the disease. Those with persistently elevated IgE levels may be the most likely to develop polyarteritis and hence the most susceptible to serious cardiovascular complications. Specific IgE antibodies may be important in the pathogenesis of the disease. These could bring about the release of chemical mediators which in turn could increase vascular permeability and facilitate antigen-antibody deposition and leukocyte migration in areas such as the arteriolar intima where the pathology chiefly occurs and where rickettsial-like bodies have reportedly been found.

Since serum IgE levels may reflect disease activity in MCLS prospective studies should be carried out in conjunction with sedimentation rates, electrocardiograms, roentgenographic studies, etc., to determine the clinical utility of serial serum IgE levels in following children during and after the acute phase of the disease.

In 1967 Kawasaki described a newly recognized disease in infants and young children which he called MCLS (9, 22). It is characterized by fever persisting for more than 5 days which is nonresponsive to antibiotics, conjunctival congestion, dry red fissured lips, reddened tongue and oral mucosa, nonpurulent lymphadenopathy and red palms and soles with late desquamation of the fingers and toes (2, 9, 10). Additional features may include diarrhea, arthralgia or arthritis, aseptic meningitis (10), carditis (2), pericarditis, aneurysmal dilation and thrombosis of coronary arteries, and sudden death (1, 8, 21). There may be proteinuria, leukocytosis, increased platelets, elevated erythrocyte sedimentation rate, a positive test for C-reactive protein, and mild jaundice (10). The disease may be mistakenly diagnosed as scarlet fever (10), Stevens-Johnson syndrome (10), or be confused with infantile polyarteritis nodosa (17, 18).

Because of the striking similarity of some cases to infantile polyarteritis nodosa (12–14), a disease recently reported to be associated with elevated serum levels of serum IgE (12), the paired acute and convalescent serum IgE levels of 20 subjects with acute nonfatal MCLS were studied along with 20 near-age controls from the same communities in Japan. The results indicate that most if not all subjects with MCLS have an elevation of IgE during the acute phase of the disease, usually peaking 1–2 weeks after onset and declining towards the control value over the ensuing 1–2 months. The purpose of this paper is to document alterations in serum IgE levels which accompany this disease and to discuss possible implications.

MATERIALS AND METHODS

Serum specimens were obtained from 20 subjects in Japan with the typical clinical manifestations of acute febrile MCLS. Initial specimens were obtained from the 5th to the 30th day of illness (mean 11.6 days from onset). Follow-up specimens were obtained from the 16th to the 58th day of illness (mean 32.9 days from onset). The mean age of the patients at onset of the disease was 30.9 months compared with a mean age of 26 months for the control subjects. The parents of all subjects were questioned regarding a past history of allergy in the child. None in either group had clinical evidence of allergy or parasitic infestation when examined or during hospitalization. After learning the results of the assays, all clinical records were again carefully reviewed by one of the authors (*SK*). Only the 46-month-old control child (IgE value 240 IU/ml) had a history of possible allergy, in this case transient urticaria associated with tonsilopharyngitis. There was no other history of urticaria, atopic dermatitis, asthma, seasonal rhinitis, food sensitivity, or other allergy in any instance.

IgE levels were performed using the double antibody technique described by Gleich *et al.* (5) with slight modification as described elsewhere (20). The use of antiserum to one E-myeloma protein (*PS*) and of radiolabeled IgE (*ND*) isolated from a second E-myeloma patient eliminated the problem of idiotypic antibodies preferentially reacting with the radiolabeled protein rather than with the IgE in the serum specimens. All tests were done in duplicate on the same day. A standard curve was constructed using carefully prepared dilutions in 1% human serum albumin of a known secondary standard. This standard was previously assayed by Dr. S. G. O. Johansson and twice by Dr. R. Hamburger. It was assayed against the current World Health Organization standard in this laboratory on several occasions and has proved to be a stable secondary standard.

Because serum IgE levels of healthy populations have been found to deviate from a normal Gaussian distribution curve and because percent changes in levels are described more meaningfully by logarithmic than arithmetic values, all IgE levels were reduced

to logarithms for statistical analysis. The Z-test was used to compare both the initial and the follow up serum IgE levels to the levels found in the control population. The *t*-test for dependent variables was used to compare the initial IgE levels to those of the matched follow-up specimens obtained from the same subjects.

RESULTS

Table 1 indicates that the geometric mean serum IgE level of the initial serum specimens (156.9 IU/ml) was significantly elevated in comparison to that of the follow up specimens (71.8 IU/ml, $P < 0.01$). Geometric mean values for both the initial and the follow-up specimens were also elevated in comparison with those of the control subjects (37.7 IU/ml), but only that for the initial specimens was significantly higher than controls, $P = 0.005$ (convalescent vs. control geometric mean, $P = 0.12$). As might be expected, IgE levels were lower in the younger subjects of both patient and control groups than in the older ones (Table 1). The youngest patient, 2 months of age, was unusual in having an increased serum IgE in the second specimen (increased from 17 to 40 IU/ml between the 6th and 16th days of illness). One of the two 6-year-olds also showed an increase in IgE level between the first (5th day) and second (38th day) specimen. All other patients showed a decline in serum IgE as convalescence progressed except for the 18-month-old whose value remained the same. A careful look at Figure 1, however, suggests the likelihood that in most, if not all, subjects the peak IgE level is reached after the 6th day of illness and that once the peak is reached there is a more gradual decline towards control levels. The fact that the mean convalescent serum level was still above that of the control group suggests that many levels may not approximate the control value until later than 33 days after onset of the disease.

Table 1. *IgE levels in acute (A) and follow-up (B) sera, and in controls, of similar place of residence*[1]

MCLS[2] patients				Control subjects	
	IgE, IU/ml				
Age, mo	Initial		Follow-up	Age, mo	IgE, IU/ml
2	17	(6[3])	40 (16[3])	2	3.5
7	[illegible]	(20)	68 (34)	2	3.5
7	[illegible]	(11)	28 (58)	4	2.0
8	[illegible]	(12)	130 (17)	7	16
14	[illegible]	(9)	4.4 (33)	8	44
16	[illegible]	(5)	8.8 (39)	11	96
16	900	(9)	460 (21)	12	15
17	40	(30)	15 (43)	12	37
17	60	(5)	25 (47)	16	38
18	34	(20)	34 (30)	16	11
24	900	(6)	620 (20)	18	28
30	22	(7)	4.2 (28)	36	700
36	180	(10)	98 (25)	36	5.2
36	560	(6)	140 (33)	36	12
50	1,800	(7)	1,200 (30)	41	800
52	800	(7)	540 (30)	41	170
58	900	(9)	560 (36)	46	240
66	15	(21)	13 (38)	48	16
72	260	(5)	360 (38)	58	225
72	280	(27)	98 (42)	60	610
Mean 30.9		(11.6)	(32.9)	26.0	
Geometric mean	156.9		71.8		37.7

[1] *P* values: initial vs. follow-up, $P < 0.01$; initial vs. controls, $P < 0.005$; follow-up vs. controls, $P = 0.12$;

[2] Mucocutaneous lymph node syndrome.

[3] Days from clinical onset of MCLS.

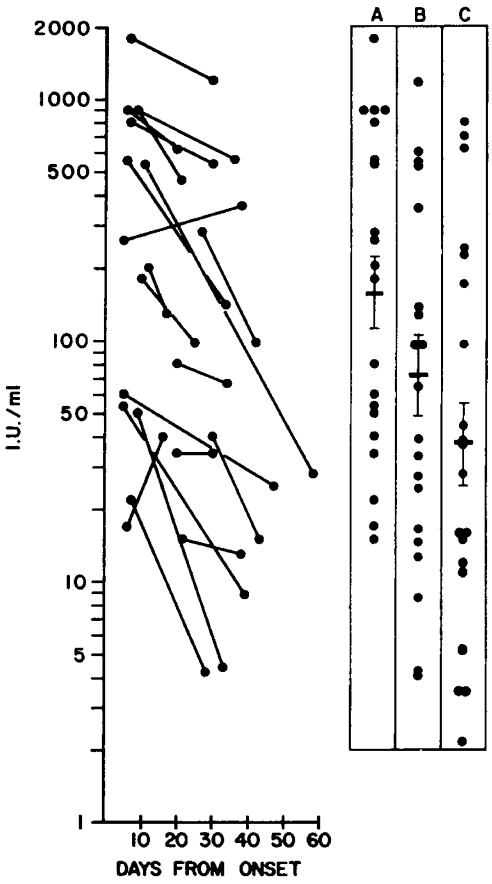

Fig. 1. Serum levels of IgE during acute and convalescent stages of mucocutaneous lymph node syndrome. *A:* initial specimens; *B:* convalescent specimens; *C:* control specimens from healthy Japanese children. Brackets indicate geometric mean ± SEM.

DISCUSSION

Infantile acute febrile MCLS is a disease which now must be added to the growing list of conditions associated with an elevation of the serum level of IgE. It may be the only acute febrile disease recognized to date which is regularly associated with a rise in serum IgE. The pattern of levels found in the 20 subjects studied suggests that the peak blood level is reached between 7 and 14 days after onset of the disease. In the average case this level is more than twice that found 1 month after onset and it is likely that it exceeds the pre-illness or recovery level by a factor of 4. The finding of a sharp increase in serum IgE in the acute stage of the disease may provide a clue to the pathogenesis of this idiopathic disease or it may simply represent a concomitant finding of little pathogenetic significance. Additional studies, particularly a search for specific IgE antibodies, may be needed to clarify this point. This of course will require better characterization of the causative agent and its availability for immunochemical studies of antibody response.

The 58-month-old patient is of special interest in that he had a typical case of acute febrile MCLS 6 months before the present illness. He appeared to recover completely, having no symptoms and enjoying excellent health in the intervening 5 months. After this, fever and other typical symptoms reappeared, and 9 days later the first blood specimen was obtained. This is one of the rare instances in which there has been a recurrence of symptoms once clinical recovery has occurred. The finding of 900 IU/ml of IgE in the serum of the 9th day of relapse and 560 IU/ml on the 36th day suggests that acute relapses are also accompanied by elevations of

IgE during the most active phase of the syndrome. Since it appears that in this disease IgE levels are a reflection of disease activity, their utility in following the progress of recovery should be evaluated.

The fact that there is a similar scatter of IgE values in patients with the disease to that of control values although at a higher level suggests that there is little if any predilection for the disease in those subjects with high pre-illness levels of IgE. This is substantiated by the lack of a personal or family history of allergy or clinical evidence of allergic disease or parasitic infestation in affected subjects or in the control population.

A careful analysis of the records of the patients in this study failed to reveal any special features characteristic of the disease in the five patients with the highest initial and follow-up IgE levels (all above 450 IU/ml). Furthermore, there were no distinguishing clinical characteristics among those whose initial and follow-up specimens had levels below 65 IU/ml. The data for all 20 patients were compatible with a brisk early rise in serum IgE during the acute phase of the illness, followed by a more gradual decline towards control values during convalescence. There is clear evidence that the serum IgE level had not yet reached its peak by the 5th or 6th day in at least two subjects, and we suspect that the peak had passed before the initial specimen was obtained from several others.

Hamashima and coworkers (6) have described the finding of rickettsia-like bodies in the skin and lymph node biopsies of 12 of 23 subjects with acute MCLS. These bodies were located in the cytoplasm of macrophages and of endothelial cells of arterioles as well as in the vascular lumen. They have reportedly been isolated in yolk sac cultures (7). Further information about this finding is awaited eagerly. If rickettsial or another microbial agent proves to be etiologic in the disease, interest in the role of IgE will not be diminished. It might be an infection in which IgE plays an important role in eliminating the invader from the body. Immunologists and allergists have long sought clear evidence for a useful role of IgE in human immunologic defense mechanisms. On the other hand, could it be that only those subjects who are genetically programmed for a brisk IgE response to this particular rickettsial-like agent will develop the full blown clinical manifestations of the disease? Clearly there will be great interest in the study of the immune responses of affected subjects and their family members once antigens become available for study. Such studies should include efforts to detect and quantitate specific antibodies of each immunoglobulin class as well as specific lymphocyte responsiveness.

An additional feature of the disease is of considerable interest. Approximately 1–2% of affected children succumb, most of whom die suddenly and are found to have myocardial infarction with coronary aneurysm and/or thrombosis at postmortem. There is a remarkable similarity in gross and microscopic postmortem findings between many fatal cases of MCLS and cases of infantile polyarteritis nodosa (4, 8, 17, 21). Also the sera of two infants, 3 and 4 months of age, who recently died of infantile polyarteritis nodosa were found to contain a high level of IgE (12). One of us (*DCH*) was asked to confirm the high levels in these cases. This was accomplished with precisely the same reagents and technique and the same standards used in the current study of MCLS. The levels in both infants were significantly elevated. Both of the infants had coronary thrombi and aneurysms and a rash similar to that seen in MCLS and one had additional clinical features of MCLS. Thus, MCLS may represent a disease which can progress to polyarteritis nodosa in infants and young children. If this is so, the fact that IgE levels were high in two fatal cases of infantile polyarteritis nodosa shortly before death indicates that in these subjects a brisk IgE response was ineffective in promoting recovery from the disease. Indeed, perhaps it suggests that IgE antibodies might contribute to the clinical features of the disease including the observed arteritis and myocarditis by increasing capillary permeability, enhancing antigen-antibody deposition, and facilitating leukocyte migration into involved areas. This would be in keeping with the role proposed for IgE in serum sickness by Benveniste *et al.* (3).

Other points perhaps worth mentioning are that Melish *et al.* (13) have reported nine cases of MCLS from Hawaii. Valaes (19) has recently described a number of cases which he believes indicates MCLS is also a newcomer in Athens, Greece. Kim *et al.* (11) have reported clinical observations on 8 cases in Korea. If these cases represent the same disease and if their increasing incidence is real, then environmental pollutants as well as new strains of microorganisms should be included in the list of possible etiologic or contributing factors. The possibility of hypersensitivity to mercury, in particular, would seem to deserve careful investigation (15). Contaminants in air, water, and food are steadily increasing around the world. One wonders if mercury or another pollutant could act as a hapten or otherwise stimulate IgE responses in genetically susceptible children, perhaps facilitating the remarkable chain of events manifested in this disease.

SUMMARY

Paired acute and convalescent serum specimens were obtained from 20 Japanese children who had the typical clinical findings of a newly recognized disease, acute febrile MCLS. There was a two- to fourfold elevation of total IgE during the acute stage of the disease without clinical evidence of allergy or other disorder which could explain it. The suggestion is made that IgE antibodies may play an important role in the pathogenesis of the disease and possibly will provide a laboratory tool for recognizing those subjects who are likely to have late cardiovascular complications.

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Bilirubin glucuronyl transferase
phenobarbital uridine diphosphate glucuronic acid

Various Bilirubin Conjugates in Pregnant and Nonpregnant Rats with and without Phenobarbital Treatment

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Extract

The relative participation of bilirubin conjugating systems other than uridine diphosphate glucuronic acid-dependent bilirubin glucuronyl transferase in 21-day pregnant (P) and nonpregmant (NP) rats with and without prior phenobarbital (PB) administration was studied. Relative enzyme activities of 74.5%, 17.6%, and 7.9% were observed for glucuronic acid, xylose, and glucose conjugates, respectively, in untreated NP rats. Pregnancy itself did not alter the relative activities. In response to PB administration, P rats increased activity less than NP animals for all three enzymes. The ratios of the three conjugates remained unchanged in both P and NP groups after PB stimulation.

Speculation

The mechanisms by which pregnancy reduces the stimulatory effect of phenobarbital on bilirubin glucuronyl transferase in the rat while not altering unstimulated enzyme activity remain unexplained.

The induced enzyme may be different from the noninduced enzyme and may, therefore, be susceptible to inhibitors present in increasing concentrations during pregnancy, inhibitors to which the noninduced enzyme is not susceptible. Alternatively, the induced enzyme may be the same as the noninduced enzyme but the reduced stimulation observed may result from either changes in phenobarbital metabolism during pregnancy or a reduced capacity to synthesize enzyme protein during pregnancy.

Phenobarbital administration to adult rats markedly increases the formation of bilirubin conjugates with glucuronic acid *in vitro*. The degree of response of maternal hepatic bilirubin glucuronide formation to PB stimulation during the second half of pregnancy has recently been found to be significantly less than in nonpregnant or early pregnant rats, despite similar hepatic phenobarbital concentrations (8). At 21 days of gestation (term) the increase in glucuronyl transferase activity was approximately one-third of that in early pregnancy (8). Although hepatic conjugation of bilirubin in mammalian species is predominantly with glucuronic acid to form the water-soluble, excretable form of bile pigment, significant quantities of glucose and xylose conjugates have also been identified in bile (1). Therefore, it was postulated that with the advance of pregnancy, there was a shift in PB-stimulated conjugation of bilirubin from predominantly that with glucuronic acid to that with xylose and/or glucose. Because our previous studies were performed only with addition of uridine diphosphate glucuronic acid to the incubation medium, relatively greater increases in glucose and xylose conjugates would have gone undetected.

The purpose of this study, therefore, was to explore the relative participation of bilirubin conjugating systems other than uridine diphosphate glucuronic acid (UDP-GA)-dependent bilirubin glucuronyl transferase in 21-day P and NP rats with and without prior PB administration.

MATERIALS AND METHODS

Sprague-Dawley 18-day P and NP female rats weighing between 270 and 340 grams were obtained from Marland Breeding Farms, N. J. The animals were handled, housed, fed, treated with PB (100 mg/kg/24 hr s.c. for 3 days) and killed in exactly the same manner as described in our previous study (8). Livers were removed immediately after time of killing, washed in cold 0.154 M KCl, blotted, and weighed. Homogenates (8%) were prepared in cold 0.154 M KCl using a Teflon pestle. Bilirubin transferase assays were done immediately using whole liver homogenates (0.1 cc) (digitonin activated) with bilirubin as substrate and either UDP-GA (10) (6.19×10^{-2} M), uridine diphosphate xylose (UDP-X) (10) (2.49, 4.98, 7.46, 14.92, and 22.38×10^{-2} M), or uridine diphosphate glucose (UDP-G) (10) (2.19, 4.38, 6.56, 13.2, 19.7, and 26.2×10^{-2} M). Optimal concentrations were established at 7.46×10^{-2} M UDP-X and 6.56×10^{-2} UDP-G and used in a micromodification of the previous method (3). Each sample was run in duplicate. Intensity of color formed was determined spectrophotometrically at 525 nm using quartz microcells with a 1-cm light path in a Perkin-Elmer double beam spectrophotometer with a final total volume of 0.92 cc. Uridine diphosphate glucuronyl transferase (UDP-GT), uridine diphosphate xylosyl