

Hypermacroglobulinemia associated with heroin use in adolescents

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Increases of serum 19S IgM globulin were detected in adolescent patients admitted for narcotic detoxification. The mean IgM level was 294 ± 130 mg. per cent compared to 164 ± 63 mg. per cent in a control group. Twenty-six of the 64 adolescent narcotic users (40.6 per cent) had IgM levels above 290 mg. per cent, the upper limit of normal. There was no evidence of cytomegalovirus infection, infectious mononucleosis, primary biliary cirrhosis, mixed cryoglobulinemia, or cold agglutinins. The presence of Australia antigen in 2 patients and elevated serum glutamic oxaloacetic transaminase (SGOT) levels in 19 suggests that the increased IgM levels may be related to the presence of chronic persistent hepatitis. Heroin use should be considered as an important cause of elevated serum IgM, especially in association with relatively normal levels of IgG and IgA.

Polyclonal increases of serum 19S are noted frequently in association with diffuse hypergammaglobulinemia.¹ Isolated elevation of these macroglobulins has been noted as well with type 1 dysgammaglobulinemia,² infectious mononucleosis,^{3, 4} African trypanosomiasis,⁵ MS-1 type viral hepatitis,⁶ primary biliary cirrhosis,⁷ and cytomegalovirus infection.⁸ In a recent study of adult patients with chronic heroin (diacetylmorphine) addiction, Nickerson and associates⁹ reported that the levels of IgA and IgG were surprisingly normal, while IgM elevations were present in 17 of 25 sera ranging from 210 to 660 mg. per cent. Positive tests in latex fixation and Ripley-coated cell agglutination were detected in 9 of 25 addicts, but no gross elevation of IgD or IgE was noted by immunoelectrophoresis.

The purpose of the present study was to examine the incidence of polyclonal hypermacroglobulinemia in adolescent heroin users and to determine whether this abnormality is associated with any of the previously recognized etiologic factors.

MATERIALS AND METHODS

Immunoglobulins

Quantitative immunoglobulin levels were determined in the sera from 64 patients admitted consecutively to the Adolescent Narcotic Addiction Service at St. Luke's Hospital Center during March and April, 1970. The subjects ranged from 14 to 23 years of age and were predominantly males (5 $\frac{5}{6}$). Sixty-two of the patients were on intravenous heroin, while in 2 the habit was limited to skin popping. Toxocologic studies detected morphine derivatives in

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the urine of 21 of 54 patients, confirming recent drug use. All sera were collected within 48 hours following admission for detoxification.

Sera were stored for 2 to 7 days at 4° C. with 0.01 per cent sodium azide. Monospecific rabbit antisera to IgG, IgA, IgM, and IgD (Behring Diagnostic, Inc., Woodbury, N. Y.) gave a single precipitin arc on immunoelectrophoresis against stored normal human serum. These antisera were used to prepare agar plates, and IgG, IgA, IgM, and IgD concentrations were measured by the semiquantitative single radial immunodiffusion technique of Mancini, Carbonara, and Heremans.¹⁰ Plexiglas plates (Melpar, Inc., Falls Church, Va.) measuring 2 by 3 inches were layered with a 10 ml. mixture containing 9 ml. 1.0 per cent agar and 1 ml. antiserum. Wells of 2.0 mm. diameter were cut, and 16 μ l of a dilution of each serum was added to the wells. The serum was undiluted for IgD and diluted 1:5 for IgM, 1:8 for IgA, and 1:40 for IgG. The plates were incubated in a high humidity chamber for 24 hours. Three dilutions of standardized and stabilized pooled human serum (Behring Diagnostic, Inc.) (from which labile proteins such as alpha-1 lipoprotein, alpha-1 antitrypsin, beta lipoprotein, and C-reactive protein have been removed) were added to 3 wells in each plate to provide the reference standard. For the IgM determination, the reference standard was undiluted and in dilutions of 1:3 and 1:6. At 24 hours the diameter of the precipitin ring was measured carefully, and values for immunoglobulins were read from a semilog curve derived from the 3 dilutions of the standard. In this laboratory normal ranges and standard deviations for these values in 50 healthy adolescents matched for sex and race were IgG = $1,244 \pm 63$ mg. per cent, IgA = 254 ± 78 mg. per cent, and IgD = 0.10 ± 0.05 mg. per cent.

Australian (Au) antigen

Au antigen was detected in serum by 2 dimensional immunodiffusion and counterelectrophoretic methods.^{11, 12} The specificity of positive reactants was confirmed by comparison with reference standards of Au antigen.

Serologic studies

Serum cytomegalovirus (CMV) antibody titers were measured by the microcomplement fixation test¹³ using CMV-infected human embryonic lung cell cultures with 90 per cent cytopathic effect as antigen and uninfected cell cultures as controls. These studies were performed by Dr. Donald Armstrong, of Memorial Hospital, New York City. Immuno-fluorescent studies for smooth muscle and mitochondrial antibodies^{14, 15} were performed with selected sera by Dr. Fenton Schaffner, Mount Sinai Hospital, New York City. Serum was refrigerated at 4° C. and observed over a period of 72 hours for the presence of cryoglobulins. Studies of cold agglutinins, heterophil antibody, VDRL, fluorescent antinuclear antibody, and latex fixation for rheumatoid factor were performed with standard methods. Serum chemistries were performed with a 12 channel autoanalyzer. Statistical analysis was conducted with the Student t test.

RESULTS

The mean quantitative serum immunoglobulin levels for the 64 adolescent narcotic users and 50 normal subjects are presented in Table I. The levels of IgG, IgA, and IgD were similar in both groups. However, the mean IgM level of 294 ± 130 mg. per cent in the drug users was significantly elevated in comparison to the control value of 164 ± 63 mg. per cent ($p < 0.005$). Twenty-six of the 64 adolescent narcotic users (40.6 per cent) had IgM levels above 290 mg. per cent, the upper limit of normal.

Tables II and III summarize the studies in the 26 patients with IgM levels above 290 mg. per cent and in the 38 subjects with IgM determinations below this level. There were no significant differences in age, sex, and race between the two groups.

TABLE I. Quantitative serum immunoglobulin levels in 64 adolescent narcotic users

Immunoglobulin	Narcotic users, 64 patients (mg. %)	Control sera, 50 subjects (mg. %)
IgG	1,425 $\pm$ 281	1,244 $\pm$ 306
IgM	294 $\pm$ 130*	164 $\pm$ 63*
IgA	192 $\pm$ 70	254 $\pm$ 78
IgD	0.12 $\pm$ 0.09	0.10 $\pm$ 0.05

*P value < 0.005 by t test.

TABLE II. Summary of studies in 26 adolescent heroin users with increased serum IgM levels*

Patient No.	Age	Sex	Race	Immunoglobulins				Chemistries			Australia anti-gen	Rheu-matoid factor
				IgM (mg. %)	IgG (mg. %)	IgA (mg. %)	IgD (mg. %)	Total bili-rubin (mg. %)	Alka-line phos-phate I.U. (N $\leq$ 85)	SGOT I.U. (N $\leq$ 50)		
1	17	M.	B.	725	1,360	368	0.19	1.0	40	48	Neg.	Neg.
2	17	M.	B.	675	2,000	232	0.27	NP	NP	NP	Neg.	Neg.
3	18	M.	B.	325	1,400	240	0.08	0.5	55	35	Neg.	Neg.
4	17	M.	B.	375	1,680	200	0.27	0.6	90	77	Neg.	NP
5	18	M.	W.	525	1,760	152	0.42	0.8	75	145	Neg.	1-400
6	17	M.	W.	375	1,240	192	0.06	0.7	40	50	Neg.	Neg.
7	16	M.	B.	310	1,320	136	0.08	0.3	70	135	Neg.	Neg.
8	18	F.	B.	410	1,760	288	0.15	0.4	50	80	Neg.	Neg.
9	18	M.	B.	450	1,680	360	0.17	0.6	160	95	Neg.	1-20
10	17	M.	B.	525	2,040	144	0.08	0.3	70	105	Neg.	1-20
11	19	M.	B.	450	1,320	216	0.06	0.9	110	65	Neg.	1-20
12	18	M.	B.	310	1,280	328	0.04	0.9	125	185	Neg.	1-200
13	18	F.	B.	310	1,280	184	0.07	0.7	60	30	Neg.	Neg.
14	18	M.	B.	310	1,600	360	0.07	1.0	50	90	Neg.	1-20
15	17	M.	W.	400	1,400	192	—	1.1	170	1,250	Pos.	Neg.
16	18	M.	B.	350	1,520	156	0.05	0.9	60	35	Neg.	Neg.
17	19	F.	W.	485	1,480	216	0.27	0.6	50	55	Neg.	Neg.
18	17	M.	W.	400	1,680	148	0.05	0.8	90	60	Neg.	Neg.
19	16	M.	W.	550	1,200	176	—	7.8	160	1,250	Pos.	Neg.
20	18	M.	W.	500	1,520	248	0.08	0.8	60	50	Neg.	Neg.
21	19	M.	B.	350	1,400	124	0.04	0.5	100	75	Neg.	Neg.
22	18	M.	B.	380	1,760	160	0.09	0.5	70	75	Neg.	1-200
23	17	M.	B.	415	1,600	216	0.06	0.7	75	70	Neg.	1-200
24	18	M.	B.	415	1,760	108	—	0.4	90	175	Neg.	Neg.
25	17	F.	W.	450	1,520	160	0.10	0.4	55	70	Neg.	Neg.
26	17	M.	W.	475	1,840	152	0.11	1.4	65	100	Neg.	Neg.
Mean	17.6	M=85%	B=65%	430.6	1,555.4	209.8	0.12	0.98	81.6	176.2		
S.D.	0.8			102.3	230.6	75.3	0.10	1.45	37.3	325.7		

NP = Not performed.

*Sera from all 26 patients were negative for indirect fluorescent antinuclear antibody, heterophil titers of 1:56 or greater, cryoglobulins, and cold agglutinins; 10 of these sera were negative for antismooth muscle and antimitochondrial antibodies, while the VDRL was positive in 1 of 23 in a titer of 1:2 and the cytomegalovirus C-F titer was positive in 1 of 7 in a low titer of 1:8.

In the group of patients with hypermacroglobulinemia, the results of measurements of IgG, IgA, and IgD serum levels were within normal limits with means of $1,555 \pm 231$, 210 ± 75 , and 0.12 ± 0.10 mg. per cent, respectively. IgG levels were above 1,856 mg. per cent, the upper limit of normal, in only 2 patients. The IgM levels in this group varied from 310 to 725 mg. per cent with a mean of 430.6 ± 102.3 mg. per cent. Immunoelectrophoretic studies with each of these

TABLE III. Summary of study in 38 adolescent heroin users with normal serum IgM levels*

Patient no.	Age	Sex	Race	Immunoglobulins				Chemistries			Australia antigen
				IgM (mg. %)	IgG (mg. %)	IgA (mg. %)	IgD (mg. %)	Total bilirubin (mg. %)	Alkaline phosphatase I.U. (N ≤ 85)	SGOT I.U. (N ≤ 50)	
1	15	M.	W.	200	1,520	272	0.44	0.6	120	45	Neg.
2	18	M.	W.	130	920	108	0.15	0.6	40	62	Neg.
3	17	M.	B.	165	1,760	216	0.15	1.2	115	255	Neg.
4	14	M.	W.	165	1,120	256	0.10	0.5	295	120	Neg.
5	17	M.	W.	130	1,120	108	0.06	1.0	85	525	Neg.
6	16	M.	B.	130	1,440	144	0.05	0.6	200	230	Neg.
7	19	M.	B.	250	1,720	272	0.07	1.1	65	100	Neg.
8	18	M.	B.	210	1,520	240	0.22	0.8	80	45	Neg.
9	17	M.	B.	250	1,240	216	0.14	0.8	50	50	Neg.
10	16	M.	B.	160	1,240	184	0.08	1.6	240	115	Neg.
11	16	M.	B.	220	1,040	132	0.12	0.5	105	38	Neg.
12	14	M.	B.	72	960	140	0.19	0.8	125	45	Neg.
13	16	M.	W.	210	1,640	104	0.08	1.0	85	185	Neg.
14	18	M.	B.	285	1,440	208	0.08	0.9	90	110	Neg.
15	16	M.	W.	195	1,280	216	0.18	0.4	100	40	Neg.
16	18	M.	B.	265	2,000	360	0.22	1.3	70	160	Neg.
17	19	M.	B.	170	1,160	168	0.04	1.0	95	80	Neg.
18	17	M.	B.	92	960	116	0.13	0.6	90	50	Neg.
19	17	M.	W.	130	1,400	256	0.06	1.8	82	175	Neg.
20	18	M.	W.	285	1,000	140	0.03	0.8	50	40	Neg.
21	18	M.	B.	115	1,440	256	0.07	0.5	55	70	Neg.
22	17	M.	B.	265	1,400	92	0.05	0.7	85	80	Neg.
23	18	M.	B.	210	1,400	116	0.14	1.1	70	95	Neg.
24	18	F.	B.	87	740	128	0.03	0.6	60	65	Neg.
25	18	F.	B.	190	1,248	144	0.08	0.7	45	50	Neg.
26	18	M.	B.	135	1,360	120	0.13	0.6	130	60	Neg.
27	18	M.	B.	290	1,220	176	0.39	1.9	65	100	Neg.
28	18	M.	B.	150	1,680	176	0.06	0.6	140	40	Neg.
29	16	M.	B.	175	1,280	168	0.04	1.3	120	140	Neg.
30	18	M.	B.	285	1,200	148	0.07	1.1	75	85	Neg.
31	16	M.	B.	175	1,600	176	0.04	0.4	30	40	Neg.
32	16	M.	W.	150	1,360	176	0.14	0.7	85	60	Neg.
33	16	M.	B.	260	1,680	336	0.28	0.8	90	45	Pos.
34	18	M.	W.	285	1,600	148	0.05	0.4	100	95	Neg.
35	18	F.	B.	82	880	128	0.04	0.4	40	50	Neg.
36	20	F.	B.	210	1,600	144	0.04	0.7	55	65	Neg.
37	23	F.	W.	285	1,040	160	0.05	0.4	80	75	Neg.
38	18	M.	B.	240	1,600	152	0.11	0.4	90	80	Neg.
Mean	17.4	M=87%	B=71%	187.2	1,337.1	178.9	0.11	0.82	94.7	99.1	
S.D.	1.6			70.8	284.1	64.4	0.08	0.38	53.0	88.5	

NP = Not performed.

*Sera from all 38 patients were negative for indirect fluorescent antinuclear antibody, heterophil titers of 1:56 or greater, cryoglobulins, cold agglutinins, and rheumatoid factor; the VDRL was positive in 1 of 24 while the cytomegalovirus titer was negative with all 3 sera tested.

sera demonstrated a smooth IgM arc, suggesting that the hypermacroglobulinemia was polyclonal. A typical study is presented in Fig. 1.

Table IV presents data comparing the narcotic use duration, cost, route of intake, and urine toxicology in the groups with and without increased IgM. The patients with hypermacroglobulinemia appeared to be more intense drug users as suggested by a greater duration and cost of narcotic intake and a higher prevalence of drug in the urine.

Australia antigen was detected in 2 of 26 hypermacroglobulinemic patients

TABLE IV. Comparison of the addiction duration, cost, route of intake, and urine toxicology in adolescents with normal and increased IgM levels

	Duration (months)	Cost per day (\$)	Route of intake		Positive urine morphine derivative/ Number of patients
			Intravenous	Skin popping	
Normal IgM	29.4 $\pm$ 15.9	21.4 $\pm$ 17.5	36	2	11/34
Increased IgM	34.2 $\pm$ 20.5	25.5 $\pm$ 17.3	26	0	10/20

as compared to 1 of 38 positive sera in the group of subjects with normal levels of IgM. Measurement of total bilirubin or alkaline phosphatase did not correlate with the presence or degree of hypermacroglobulinemia. Serum glutamic oxaloacetic transaminase (SGOT) levels were elevated in 19 of 25 patients with increased serum IgM and in 26 of 38 with normal IgM.

Cytomegalovirus complement fixation titer was 1 : 8 in one of seven patients tested with IgM elevation and was negative in 3 subjects with normal levels of the immunoglobulin. The VDRL was positive in low titer in only one patient with elevated IgM, and heterophil titers of 1 : 56 or greater were not detected. Antinuclear, antismooth muscle, and antimitochondrial antibody were not demonstrated.

Antigamma globulin activity by latex fixation was detected in 8 of 25 hypermacroglobulinemia subjects with titers ranging from 1 : 20 to 1 : 400 but was not detected in any of the subjects with normal IgM levels. Heat inactivation of the serum at 56° C. for 30 minutes did not result in decreased titers of rheumatoid factor. Cold agglutinin titers greater than 1 : 4 and cryoglobulins were not noted in the serum of any patient with hypermacroglobulinemia.

DISCUSSION

Serum IgM monoclonal elevations are characteristic of Waldenström's macroglobulinemia but may be associated with malignant lymphomas¹⁶ and asymptomatic relatives of patients with Waldenström's macroglobulinemia.¹⁷ Polyclonal elevation of IgM has been found commonly in association with diffuse hypergammaglobulinemia occurring with hepatic, collagen, and infectious diseases.¹ With diffuse hypergammaglobulinemia, IgA and IgG increases are noted more frequently than are elevations of IgM.¹⁸ This pattern is characteristic of diseases such as systemic lupus erythematosus,¹⁹ rheumatoid arthritis,^{20, 21} sarcoidosis,²² and tuberculosis.²²

Polyclonal increase of IgM in association with diminished levels of IgA and IgG is found typically in dysgammaglobulinemia type 1.^{2, 23} Predominant polyclonal increases of IgM with less elevation of IgG and IgA usually have been associated with viral and parasitic infections, including infectious mononucleosis and cytomegalovirus infection.²⁴⁻²⁸ In this group of 26 adolescent narcotic users with polyclonal IgM elevation, there was no serologic evidence of either of these infections. In addition, cryoglobulins and cold agglutinins that are associated with both diseases were not detected.

Hepatitis represents a third viral infection associated with IgM elevation. Studies in children by Giles and Krugman⁶ revealed that short incubation period

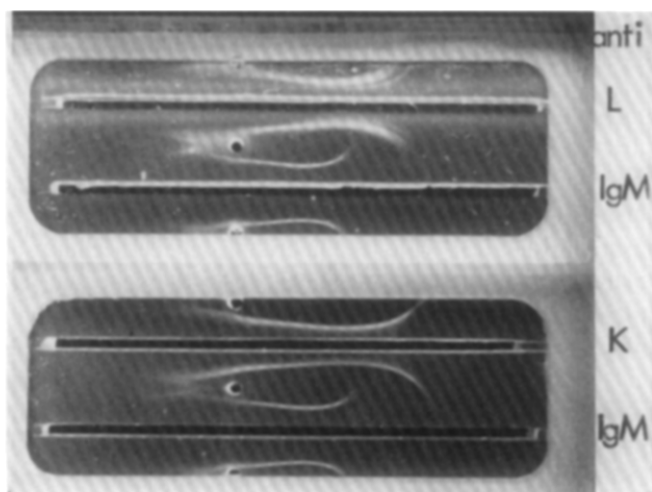


FIG. 1. The polyclonal nature of the hypermacroglobulinemia is suggested by the smooth IgM arc on immunoelectrophoresis with rabbit anti-IgM. Anti-k and anti- λ antisera do not reveal a monoclonal increase in the IgM region.

hepatitis (MS-1) is characterized by a high increase in IgM levels occurring 3 to 4 days after the first evidence of abnormal SGOT activity. The elevated IgM levels returned to normal 5 to 35 days later. In contrast, long incubation period hepatitis (MS-2), associated with circulating Australia/SH antigen,²⁹ was usually characterized by only slightly elevated to normal IgM levels during the acute stage of the disease. Some patients with chronic persistent hepatitis are reported to have elevated serum IgM concentrations for several months following the acute phase.^{30, 31} The development of chronic aggressive hepatitis is associated more frequently with increased IgG levels³¹ and with smooth muscle antibodies detectable by immunofluorescence in about 70 per cent of cases.³² Several groups of workers have reported increased serum concentrations of IgM in patients with primary biliary cirrhosis.^{7, 33} Patients with this disease are characterized also by the presence of mitochondrial antibody in up to 94 per cent of cases.³²

In our group of patients with IgM elevation, abnormally increased SGOT levels were noted in 76 per cent and suggested the presence of hepatic inflammation, but the degree of IgM increase was unrelated to the enzyme measurement. Australia antigen was detected in the sera of 2 of 26 hypermacroglobulinemic patients, and in one of 38 with normal levels of IgM. This incidence is similar to the 4.2 per cent determined by Sutnick and associates³⁴ in 215 "normal addicts" and the 2.2 per cent reported by Cherubin, Hargrove, and Prince.³⁵ Further studies with the more sensitive radioimmunoassay technique will be required to evaluate the possible association of increased IgM and Australia antigen. The failure to detect smooth muscle and mitochondrial antibodies suggests that we are not dealing with chronic aggressive hepatitis or biliary cirrhosis. However, chronic persistent hepatitis can be ruled out only by morphologic study. Persistence of Australia antigen has been demonstrated in some cases of hepatitis,³⁶⁻³⁸ and this association is likely in drug users as

well. The presence of IgM elevation may indicate that chronic hepatitis associated with Au persistence and drug use is chronic persistent rather than chronic aggressive.

Although most of the usual antibacterial and antiviral antibodies are associated with IgG globulin, "early" antibodies are usually present in the IgM fraction. However, rises of specific 19S antibodies do not usually account for quantitative serum IgM elevation. There is no evidence in this study to suggest that mixed cryoglobulins, 19S cold agglutinins, or Wassermann antibody contribute to the macroglobulinemia in heroin users. Nickerson and associates⁹ have demonstrated striking increments in heat-stable opsonins for *Escherichia coli* and *Serratia marcescens* in the majority of sera from 25 addicts when compared to normal controls. Sucrose density gradient ultracentrifugation demonstrated 19S as well as 7S antibacterial opsonins, the former correlating with IgM levels. In addition, rheumatoid factor was demonstrated in 36 per cent of their addicts. Similarly, rheumatoid factor was noted in 32 per cent of our hypermacroglobulinemic patients but not in our subjects with normal IgM levels. The failure of heat inactivation to decrease latex agglutination titers for rheumatoid factor suggests that Clq interference was not significant.³⁹ Specific antibody may be the result of multiple antigenic exposures from unsterile injection practices and adulterants. Particulate antigens and gram-negative bacterial toxins have been shown to stimulate IgM antibody production. Ryan, Parker, and Williams⁴⁰ demonstrated binding of tritium-labeled morphine sulfate to the 7S fraction of 40 per cent of heroin addicts, but no evidence was found of binding to IgM globulins.

It is possible that the quantitative IgM increase does not represent a response to specific antigens but may result from nonspecific stimulation from adulterants and additives that include quinine, lactose, mannitol, baking soda, and mannite (mannitol, dextrin, sucrose, fraxin, and gum resin). It is also possible that the persistent IgM increase may be the result of decreased catabolism (normal IgM half-life has recently been estimated at 10.1 days).⁴¹

The presence of IgM heavy chain fragments leading to false elevation of the quantitative IgM level is unlikely in view of the smooth IgM arc on immunoelectrophoresis and absence of a more anodically moving fraction reacting with anti-IgM sera (Fig. 1).

While the nature of the polyclonal IgM elevation noted previously in 68 per cent of adult heroin addicts,⁹ and here in 41 per cent of adolescent drug users, remains incompletely understood, this characteristic immunoglobulin change should be considered among the implications of quantitative increases of serum IgM globulin. Narcotic use certainly is one of the most common causes of this finding in our hospital, which is located in a large urban area.

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