Light-Chain Heterogeneity of Cold Agglutinins

Abstract. *Cold agglutinins with specificity to I or to i antigens of human-adult or cord-blood erythrocytes produced during the course of* Mycoplasma pneumoniae *infection and infectious mononucleosis contain light chains of K and L types. However, cold agglutinin isolated from the serums of patients with chronic cold-agglutinin hemolytic anemia contains only type K light chains. The experimental evidence suggests that some cold agglutinins contain both types of light chains in the same molecule.*

Cold agglutinins which react at low temperature with erythrocyte antigens designated by Wiener *et al.* as I and i (*1*) belong to the macroglobulin class of immunoglobulin (IgM) (*2*). Immunoglobulins have been found to be of either the K or L type, depending on the immunologic characteristics of their light polypeptide chains (*3*). Approximately two thirds of the IgM molecules of normal serum are said to contain antigenic determinants characteristic of K light chains and one third are of type L. Cold agglutinins with specificity to I, isolated from the serums of 59 patients with chronic cold agglutinin disease, contained only K type light chains (*5*).

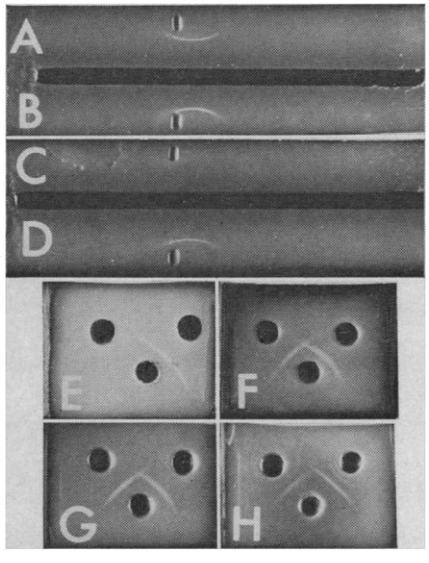

Fig. 1. *A* to *D*, Immunoelectrophoresis of eluted cold agglutinins with antiserum to human globulin. *E* to *H*, Light-chain typing of cold agglutinins by immunodiffusion against antiserums to L (left upper well) and to K (right upper well). *A* and *E*, from a patient with chronic cold-agglutinin disease; *B* and *F*, from a normal subject; *C* and *G*, from a patient with *Mycoplasma pneumoniae* infection; *D* and *H*, from a patient with infectious mononucleosis.

We have determined the immunologic type of the light chains of cold antibodies to I (naturally occurring cold agglutinin) from ten normal individuals, from five patients with chronic cold-agglutinin disease, and from 15 patients with *Mycoplasma pneumoniae* infections. Cold agglutinin with specificity to i, found in 7 of 30 consecutive patients with infectious mononucleosis, was studied in a similar manner (*6*). Cold agglutinin to I was obtained by incubation of antibody-containing serums with either compatible or autologous erythrocytes at 4°C for 2 hours and subsequent thermal elution of the antibody from the sensitized and thoroughly washed erythrocytes. Cold agglutinin to i was similarly prepared by incubation of group O erythrocytes from cord blood with serums from patients with infectious mononucleosis. All eluates were concentrated by vacuum dialysis, and the concentrates were subjected to immunoelectrophoresis. The developing antiserums had been prepared in rabbits by immunization with human IgM or with whole human serum. The eluates contained only IgM (Fig. 1).

Typing of the light chains of IgM was performed by immunodiffusion against specific rabbit antiserums to K and L. The immunodiffusion plates were incubated at 37°C for 24 to 48 hours. Prolongation of the incubation period up to 96 hours did not change the patterns obtained in 48 hours even if the amount of antiserum or eluate (or both) was increased. Cold agglutinin to I, isolated from five patients with chronic cold-agglutinin disease, contained only K light chains (Table 1, Fig. 1). Three of these patients developed this type of autoimmune hemolytic anemia during the course of malignant lymphoma. Two of these three patients had macroglobulinemia. Their serums contained 59 and 21 mg of IgM per milliliter as determined by the quantitative immunoprecipitin reaction (*7*). The IgM could be completely removed from the serum of one of these patients by successive absorptions of the antibody onto group O erythrocytes at 4°C, while only 45 percent of the IgM of the other patient could be absorbed. Thus more than half of this second patient's IgM had no cold-agglutinin activity, but both the reactive and nonreactive IgM contained only light chains of the K type.

Table 1. Reciprocal titers and immunological types of cold agglutinins.

Serums (No.)	Titers		IGM type
	To I	To i	
	Normal		
10	4–32	0–2	K, L
	Mycoplasma pneumoniae *infections*		
15	256–2048	4–128	K, L
	Infectious mononucleosis		
7	2–32	40–2048	K, L
	Chronic cold-agglutinin disease		
5	256–256,000	128–2560	K

Table 2. Reciprocal titers in the supernatant after precipitation with antiserums to K or L.

Antiserums to	Residual titer
M. pneumoniae *infections*	
0	5,120
K	40
L	40
Chronic cold agglutinin disease	
0	10,240
K	20
L	10,240

Serums of patients with pneumonia due to *M. pneumoniae* contained cold agglutinins of both K and L types (Table 1, Fig. 1). Both light-chain types were also detected in naturally occurring cold agglutinins. The seven serums of patients with infectious mononucleosis contained cold agglutinins specific to i with titers of 1:40 or greater when tested with group O cord-blood erythrocytes (Table 1). This antibody contained both IgM types (Table 1, Fig. 1). We have also found that experimentally produced cold agglutinins (*8*) also contain heterogeneous light chains.

An attempt was made to separate from an eluate, obtained from a patient with *M. pneumoniae* infection, the two types of cold agglutinins by selective precipitation with antiserums to K or L chains (Table 2). Cold-agglutinin activity of this eluate was markedly reduced or abolished after precipitation with antiserums to either light chain, while the antiserum to L did not affect the cold-agglutinin activity of eluates, obtained from patients with chronic cold-agglutinin disease, which contained only K light chains. An eluate from red cells which have been sensitized with cold agglutinin obtained from a patient with *M. pneumoniae* in-

fection was labeled with iodine-125 (*9*). This labeled eluate was incubated with antiserums to K, L, and IgM. Each of these three antiserums precipitated 90 to 95 percent of the labeled IgM. These experiments suggest that cold agglutinins obtained from patients with *M. pneumoniae* infections contain K and L determinants in the same molecule (*10*).

Cold agglutinins exclusively of L type have not yet been found. It is, therefore, possible that, in cold agglutinins containing both types of light chains, cold reactivity depends upon the presence of the type K chain. The consistency of the finding of cold agglutinins of the K type in the 59 patients with chronic cold-agglutinin disease which have been reported (*5*), and in our patients, favors the hypothesis that these monotypic antibodies may be the product of single clones of malignant cells.

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10. The finding of two different light-chain types in the same cold agglutinin molecule is in apparent contradiction to the immunodiffusion pattern of nonidentity (Fig. 1*G*). The contradiction is best explained by the heterogeneity of the molecules in the antigen well. IgM is a polymer, probably a pentamer of 7*S* subunits which contain two heavy and two light chains [F. Miller and M. Metzger, *J. Biol. Chem.* **240**, 4740 (1965)]. The ratio of K to L chains in these molecules may vary and thus produce immunodiffusion patterns of nonidentity. Similar observations have been made by W. E. Paul and B. Benacerraf, *J. Immunol.* **95**, 1079 (1965), and also discussed by A. J. Crowle, *Immunodiffusion* (Academic Press, New York, 1961), pp. 75–77.
11. We thank Dr. J. P. Griffin from the U.S. Great Lakes Naval Hospital, Great Lakes, Ill., for supplying the serums of patients with *M. pneumoniae*; Dr. Lee from the Mount Sinai Hospital, Chicago, Ill., for the serums of patients with infectious mononucleosis; and L. Hall for technical assistance.

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Sweating Exercise Stimulation during Circulatory Arrest

Abstract. *We have direct evidence of neurogenic stimulation of human exocrine sweating by muscular exercise. Sweating was markedly increased when warmed venous blood was prevented from reaching the heat-loss center in the hypothalamus.*

There is an augmentation of sweating within 1.5 to 2 seconds after the initiation of heavy muscular work by human subjects in a warm environment (*1*). The stimulus for this increase of sweating during the first few seconds of work appeared to be nonthermal, as warmed blood from the working muscles could not have reached the centers which regulate heat dissipation. From this observation we concluded indirectly that certain sweating responses during exercise in a warm environment can be independent of changes in central temperature.

Direct evidence of neurogenic stimulation of the sweating mechanism during muscular exercise has now been obtained from experiments in which the return of warmed venous blood to the heat-loss centers was prevented by arterial occlusion. Three healthy young adult male subjects performed isometric contractions against a stiff elastic cord while the circulation to and from the working muscles was arrested by an inflated pneumatic cuff which was placed high around the upper arm. With the elbow remaining on the arm rest of a chair, the flexor group of the arm musculature was contracted, the forearm being raised approximately 30° above its resting position. The subjects made no additional movements during the arm-muscle contractions, which lasted 75 seconds (Fig. 1) and 120 seconds (Fig. 2) in different experiments. The sweating rates were continuously and simultaneously recorded from small skin areas of nonoccluded limbs by the method of resistance hygrometry (*2*).

Figure 1, *B* and *D*, shows that, while venous blood warmed by the working muscles could not activate the heat-loss center in the hypothalamus for at least 75 seconds, the rate of sweating increased 3 to 4 times above the sweating rate during rest. Some participation of pain fibers during the ischemic muscular contractions cannot be ruled out. However, the perception of pain was not apparent during the first 35 or 40 seconds, when the rates of sweating had increased 2.5 to 3 times over the resting values.

It has been suggested that thermoreceptors in the muscles, or in the veins

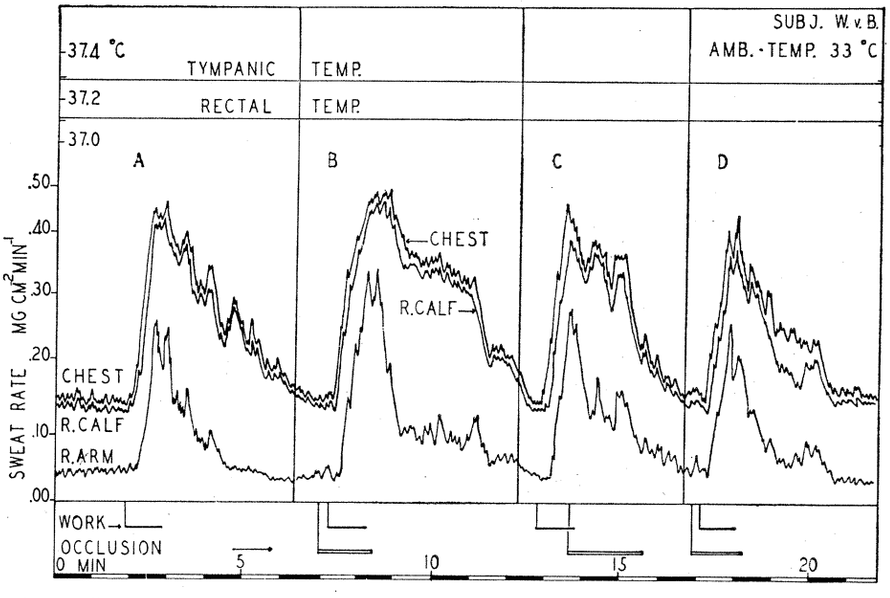

Fig. 1. Continuous recordings of sweating from three different skin areas during isometric exercise with the left arm. Experiment *A*: no occlusion. Experiments *B* and *D*: occlusion on left arm during contraction. Experiment *C*: occlusion on left arm after muscular exercise. Work, isometric contractions; occlusion, 200 mm-Hg.