Lambda Chains in Cold Agglutinins

Abstract. *Type L light chains have been found in the cold agglutinins in the serums of three patients with chronic hemolytic anemia. Although two of these cold agglutinins are unusual in that they do not have specificity to I or i red-cell antigens (one of these has, in addition, cryoglobulin property), the third is a cold agglutinin of I specificity. Previously reported cold agglutinins from such patients have all been of type K; this is the first report of type L cold agglutinins associated with chronic hemolytic anemia.*

In autoimmune hemolytic anemia associated with high-titer cold agglutinins the antibody to red cells has, in the majority of instances, a specificity for the I antigen on human red cells (*1*). The term anti-i has been applied to cold hemagglutinins which react better with i cells than with I cells (*2*). Recently, cold agglutinins which show neither I nor i specificity have been described and, for the time being, termed anti-"not-I" (*3*). So far, all cold agglutinins from serums of patients with chronic hemolytic anemia (CHA) have been reported to be macroglobulins with type K light chains exclusively (*4*). So far, no cold agglutinins with exclusively λ-chains (that is, type L light chains) have been reported. It has been suggested that the κ-chain may be related to the I specificity and to the characteristic low-temperature activity of cold agglutinins (*4*). However, the finding of κ- and λ-chains in transient cold agglutinins of I specificity, which are associated with *Mycoplasma pneumoniae* infection, has indicated that the presence of λ-chains does not preclude I specificity or low-temperature activity (*5*, *6*). Transient cold agglutinins of i specificity which are associated with glandular fever have been reported to contain both types of light chains (*6*). There are apparently no reports on the structure of CHA cold agglutinins with other than I specificity. In this report, three type L cold agglutinins from CHA patients are described: one of these having specificity to I, and the other two with specificity to the third factor ("not-I").

Case A was a 60-year-old female with CHA associated with high-titer cold agglutinins and cryoglobulinemia. Cases B and C had CHA with high-titer cold agglutinins, but cryoglobulins were absent.

Cryoglobulin was isolated from case A by repeated alternate precipitation at 1°C and redissolving at 37°C in normal saline. The serum (10 ml) was incubated in melting ice for 24 hours, and, after centrifugation at 3000 rev/min, at 1°C, for 30 minutes, the supernatant was removed, and the cryoprecipitate was dissolved in 80 ml of normal saline at 37°C. The cryoglobulin was precipitated and dissolved twice again, and after the fourth precipitation dissolved in 10 ml or normal saline.

Cold agglutinin was isolated from the three serums by repeated absorption onto and elution from type OI red cells. The serums were diluted 1:20 and inactivated by incubation at 56°C for 30 minutes. To one volume of diluted serum, half a volume of washed, packed group OI red cells was added at 37°C; the mixture was then incubated at 4°C for 45 minutes. With cases B and C, the serum was removed after centrifugation, at 4°C, for 4 minutes at 1500 rev/min. With case A no centrifugation was required owing to the rapid sedimentation of red cells. Two volumes of normal saline were added to the packed, cold agglutinin-coated red cells; the cells and normal saline were well mixed after incubation at 37°C for 15 minutes and then cooled to 4°C for 45 minutes. This absorption and elution procedure was repeated once; after the fourth absorption at 4°C the cold agglutinin was eluted at 37°C into a volume of normal saline equal to the original volume of serum, and the red cells were separated from the eluate by centrifugation at 37°C for 10 minutes, at 3000 rev/min.

Cold agglutinin titers of the serums and of the isolated antibodies were estimated by making serial twofold dilutions of 0.05 ml (with 0.025-ml Pasteur pipettes) and adding to each dilution an equal volume of a 1 percent suspension of OI, Oi, or Oi(cord) cells as required. After incubation at 4°C for 2 hours, agglutination was assessed microscopically on slides cooled to 4°C. Cold agglutinin titer was taken as the reciprocal of the highest dilution of serum or eluate in which there were agglutinates of 4 to 8 cells.

Immunoglobulin M (IgM) concentrations were determined by the ring immunodiffusion. (The immunoplates and standards were obtained from Hyland Laboratories.)

Cryoglobulin and cold agglutinin isolates were typed by immunodiffusion (Ouchterlony technique) in agar plates with wells 2 mm in diameter and 7 mm apart (center to center). Specific antiserums against IgG, IgA, and IgM were obtained from Netherlands Red Cross Laboratories and specific antiserums to κ- and λ-chains were provided by Dr. J. Fahey. The antigen wells were filled once with the cryoglobulin solution and cold agglutinin isolate from case A and the cold agglutinin isolate from case C; they were refilled four times with the more dilute cold agglutinin isolate from case B. The plates were inspected at 24 hours and daily for up to 10 days.

Case A: The serum from this patient (collected and separated at 37°C) had a cold agglutinin titer of 8192 when tested with OI, Oi, and Oi(cord) cells. As with cold agglutinins of the third variety (*4*) there was no agglutination when the red cells were previously treated with trypsin. When the cryoglobulin was removed from the serum, there was a 32-fold reduction in cold agglutinin titer, the bulk of

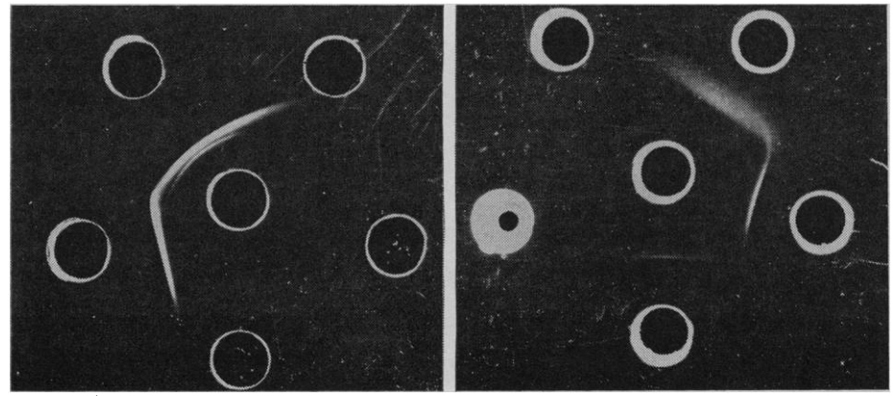

Fig. 1. Ouchterlony tests showing λ-chains in isolated cold agglutinins from cases A and C and κ-chains in a typical cold agglutinin of I specificity. The central well contained antiserum to κ-chains (left) and antiserum to λ-chains (right). Upper left, Bence Jones κ; upper right, Bence Jones λ; left of center, typical CHA cold agglutinin; right of center, cold agglutinin from case A; lower center, cold agglutinin from case C.

Table 1. Amounts of immunoglobulin M and titers of cold agglutinins in the serum fractions of case A.

	IgM (mg/100 ml)	Cold agglutinin titer
Original serum	1350	8192
Supernatant*	1090	128
Cryoglobulin solution	110	4096
Cold agglutinin eluate	110	64

* Serum supernatant after separation of cryoglobulin.

cold agglutinin activity being in the cryoglobulin solution. The IgM concentration and cold-agglutinin titers of the various fractions from this patient are summarized in Table 1. The cryoglobulin solution contained the greatest cold agglutinin activity per milligram of IgM. The reason for differences in the ratios of cold agglutinin titer to IgM level in the various fractions is not yet known (*7*). In the Ouchterlony precipitation tests with the cryoglobulin solution and the isolated cold agglutinin, precipitin lines were obtained with antibody to IgM and antiserum to λ-chain only. No reaction was observed with antiserums against IgG, IgA, and κ-chains. The antiserums to κ-chain and λ-chain were each tested with Bence Jones proteins of type K and type L at concentrations of 0.2 mg/ml. Precipitin lines were obtained with homologous antigen and antibody only (Fig. 1). On account of the unexpected finding of λ-chains, the Ouchterlony precipitation tests were repeated with two different antierums to κ- and λ-chains (provided by Dr. D. Rowe); again precipitation with only the antiserum to λ occurred. The IgM in the cold agglutinin eluate was precipitable at 1°C.

Case B: A small quantity of serum was made available (by Dr. S. Worlledge) from a patient with the CHA syndrome in whom the cold agglutinin was of the third variety as defined above. There was no cryoglobulin in this serum which had a cold agglutinin titer of 256 at 4°C and an IgM concentration of 320 mg/100 ml. The isolated cold agglutinin which had an IgM concentration of 10 mg/100 ml and a cold agglutinin titer of 16 at 4°C was typed with specific antiserums as above. Precipitation lines exclusively with antiserum to λ and antiserum to IgM were obtained.

The serum of case C had a cold agglutinin titer of 16,000 with OI cells and a titer of 2 with Oi cells at 4°C; at 20°C the respective titers were 256 and less than 2; the antibody was therefore of I specificity; the serum IgM concentration was 920 mg/100 ml. The isolated cold agglutinin had an IgM concentration of 110 mg/100 ml and a cold agglutinin titer of 2048 at 4°C. In Ouchterlony precipitation tests of this eluate, precipitation lines were obtained only with antiserum to λ and to IgM (Fig. 1).

With Dr. Fahey's antiserums to κ- and λ-chains and antiserums against IgG, IgA, and IgM, 21 more cold agglutinins of I specificity in hemolytic anemia patients have been typed and found to be macroglobulins; κ-chains only were detected in all but one (three of them associated with *Mycoplasma pneumoniae* infection); both types of light chain were found in the remaining one, associated with *M. pneumoniae* (*8*). One of these is shown in Fig. 1. The findings in cases A, B, and C indicate that cold agglutinin activity can occur in the exclusive presence of λ-chains. The cold agglutinins in cases A and B were unusual in being of the third variety and there was the additional finding of cryoglobulin in case A. The cold agglutinin in case C, however, was entirely typical in showing specificity to I. It appears, therefore, that λ-chains in the absence of κ-chains can be associated not only with cold agglutinin activity but occasionally with specificity to I as well.

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References and Notes

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7. The possibility has been considered that the cryoglobulin might be causing red-cell agglutination by virtue of its precipitation in the cold. Another type L cryomacroglobulin (provided by Professer S. Cohen) was therefore tested for cold agglutinin activity with negative findings. Cryoglobulin property is not necessarily associated with red cell agglutinating property.
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9. I thank Dr. H. Davis for the opportunity of studying case A and Professor J. V. Dacie and Dr. S. Worlledge for the serums from cases B and C. I thank Dr. W. J. Jenkins and W. L. Marsh for the antibody specificity studies of the cold agglutinin from case A. For the specific antiserums to κ- and λ-chains, I thank Drs. J. Fahey and D. Rowe. Supported by grants from the Royal Free Hospital and the Medical Research Council (U.K.)

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Disulfur Monoxide: Production by Desulfovibrio

Abstract. Desulfovibrio desulfuricans *growing on agar surfaces produces a gas that appears to be identical to "Schenk's sulfur monoxide," which was later identified as disulfur monoxide. The gas stimulates surface growth of* Desulfovibrio *on an agar medium and is used by the cells as an electron donor for the reduction of benzyl viologen.*

Desulfovibrio desulfuricans (Midcontinent strain A) was found to produce a volatile substance or gas during cell growth of this organism on plates of trypticase soy broth plus 2 percent agar (*1*). This gas was characterized by its ability to stimulate growth of the same organism on agar plates of American Petroleum Institute (API) medium (*2*) where growth was ordinarily very poor, and also by its ability to be used by the organism as an electron donor for the reduction of benzyl viologen (BV). Violet (reduced) areas of the dye were produced in agar [2 percent agar plus 0.01*M* tris(hydroxymethyl)aminomethane-HCl buffer, *p*H 7.0 ± 1, and 0.01 percent BV] around heavy concentrations of *Desulfovibrio* cells when petri plates of the agar were placed in contact with this gas in a helium or nitrogen atmosphere.

Attempts to identify this gas by gas chromatography were unsuccessful. When the volatile material was removed—by flushing with helium—from a Brewer jar containing inoculated trypticase soy agar plates and was passed through a series of dry glass traps (cooled with a mixture of NaCl and ice, a mixture of methanol and dry ice, and liquid nitrogen, successively), the gas, as indicated by the two biological properties described above, could be concentrated in the liquid-nitrogen trap. An odor resembling that of H_2S was detected in the liquid-nitrogen trap as well as in the outlet tube from this trap, which was immersed in water. The water produced blackening of lead acetate paper. The odor of H_2S could be detected, however, only infrequently from a Brewer jar containing inoculated plates of trypticase soy broth plus agar. A sample of the gas from the trap in liquid nitrogn was analyzed in the mass spectrometer, but no sulfur compounds could be detected. Substances with mass peaks of 44, 28, and 16, corresponding to CO_2^-, N_2^-, or CO^- and CH_4^-, were detected. Car-