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Research Article

AUTOHEMAGGLUTININS[1]—"COLD AGGLUTININS"

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Until cold agglutinins were reported in the serum of primary interstitial pneumonia patients (1), the phenomenon was considered rare. These findings were promptly confirmed by investigators who also observed low titers for cold agglutinins in respiratory infections without pneumonia (2 to 4). Cold agglutination was first accurately described in 1903 (5, 6) and was first found in association with bronchopneumonia in 1918 (7). Except for trypanosomiasis (8), primary interstital pneumonia is the only reported condition commonly associated with cold agglutinins. Single or small groups of cases of a variety of other diseases have been found to have cold agglutinins. Among these diseases are Laennec's (9) and syphilitic (10, 11) cirrhosis of the liver, hemolytic anemia (12, 13), paroxysmal hemoglobinuria (14, 15), peripheral vascular disease (16), benzene poisoning (17), pneumonia of unusual type (18), infectious mononucleosis (13), pernicious anemia, hyperproteinemia, and severe pneumonias (11). Of practical as well as etiological interest is the patient with cold agglutinins (19) who developed symmetrical gangrene of the fingers and toes following exposure to cold. Cold agglutinins have also been found in cats (20) and have been produced experimentally in rabbits by blood letting (21 to 24). The agglutinin is a globulin (7) which moves with the gamma groups on electrophoretic analysis (25).

The investigation of an institutional outbreak of atypical pneumonia and epidemically related respiratory infections was in progress (26) when the presence of cold agglutinins in the sera of atypical pneumonia patients was reported (1). Because the cold agglutinin test offered a laboratory means of extending our observations on the relationship between atypical pneumonias and common respiratory infections, the present study was undertaken. Patients with a number of other illnesses, acute and chronic, and a group of normal subjects were also studied for control purposes. A report on the occurrence of cold agglutinins in normal subjects, in persons with respiratory infections, and in a number of general hospital patients follows.

METHODS

Collection of blood. The stability of cold agglutinins is influenced by the method of collection and storage. We found that uniform results were best obtained when blood was drawn under sterile conditions, allowed to stand at room temperature until the clot retracted (4 to 24 hours) in order not to adsorb agglutinins on the patients' cells, and the separated serum stored aseptically at 4° C. in a tightly stoppered tube. The highest titers were obtained when the blood was tested on the day collected. The titer fell gradually over the first 3 weeks and more rapidly when the serum was repeatedly warmed for sampling. Serum stored at —70° C. in sealed glass ampoules maintained its titer for 6 months.

The agglutination test. Serial dilutions (1:5, 1:10, 1:20, etc.) of serum in 0.2 ml. of saline were mixed with 0.2 ml. of an 0.5 per cent suspension of freshly drawn, 3 times washed, normal group O human red cells (same donor throughout) and stored for 18 hours at 5° C. Shorter periods of chilling and higher temperatures did not give as uniform results. Readings were made by shaking the tube 3 times, firmly enough to make a silk-like suspension in negative tests. Agglutination was observed with the unaided eye before a 40-watt light bulb. The degree of agglutination was recorded as 1 to 5, 1 representing just visible agglutination and 5, a solid clump of cells. All tests were warmed, and the reversibility of agglutination confirmed. The results were recorded as serum dilutions. The titers reported are 1+ end-points. Since the titers regularly show an average fall of 1+ per tube as the end-point is reached and since prozones were not observed, results were uniform. Where serial tests were done on individual patients every 2 or 3 days through the course of an infection, the end-points rose and fell in smooth curves. Tests were done in groups of 20 to 70 in order properly to relate known positives and negatives to the results of the previous test on the same patient.

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RESULTS

Cold agglutinins in normal subjects. In March, April, June, and July of 1943, groups of 25 student nurses were found to have no cold agglutinins.

In September of 1943 (Figure 1), serum for cold agglutinins was drawn from 27 student nurses, 2 weeks after they first entered training. This was done at the time of their admission physical examination and x-ray of the chest. No one of these had had atypical pneumonia. One nurse gave a history of a "cold," 2 weeks before. One nurse had a chronic sore throat. Each of these had a cold agglutinin titer of 1:40. Five nurses had fresh "colds" or "coughs"; none of these had cold agglutinins. Twenty nurses were well; 5 had cold agglutinin titers, 4, 1:10, and one, 1:40. During September, October, and November, the 20 well nurses developed 17 respiratory infections, and the nurse with the sore throat also had a cold. Four of the 5 nurses who had cold agglutinins without a respiratory infection when first tested subsequently developed coughs or colds. The fifth remained well, maintaining a cold agglutinin titer of 1:40 throughout the fall, later returning to negative. Of the group with fresh respiratory infections and of those who subsequently had respiratory infections, 4 out of 5 developed a cold agglutinin titer of 1:5 or more (Table I). In November, 10 of the 27 had or were recovering from a cold or cough. Six of the 10 had cold agglutinin titers; 3 were 1:5, 1 was 1:10, and 2 were 1:40. Individuals with cold agglutinins during an infection have since gradually returned toward negative. On the other hand, the nurse with the chronic low-grade sore throat

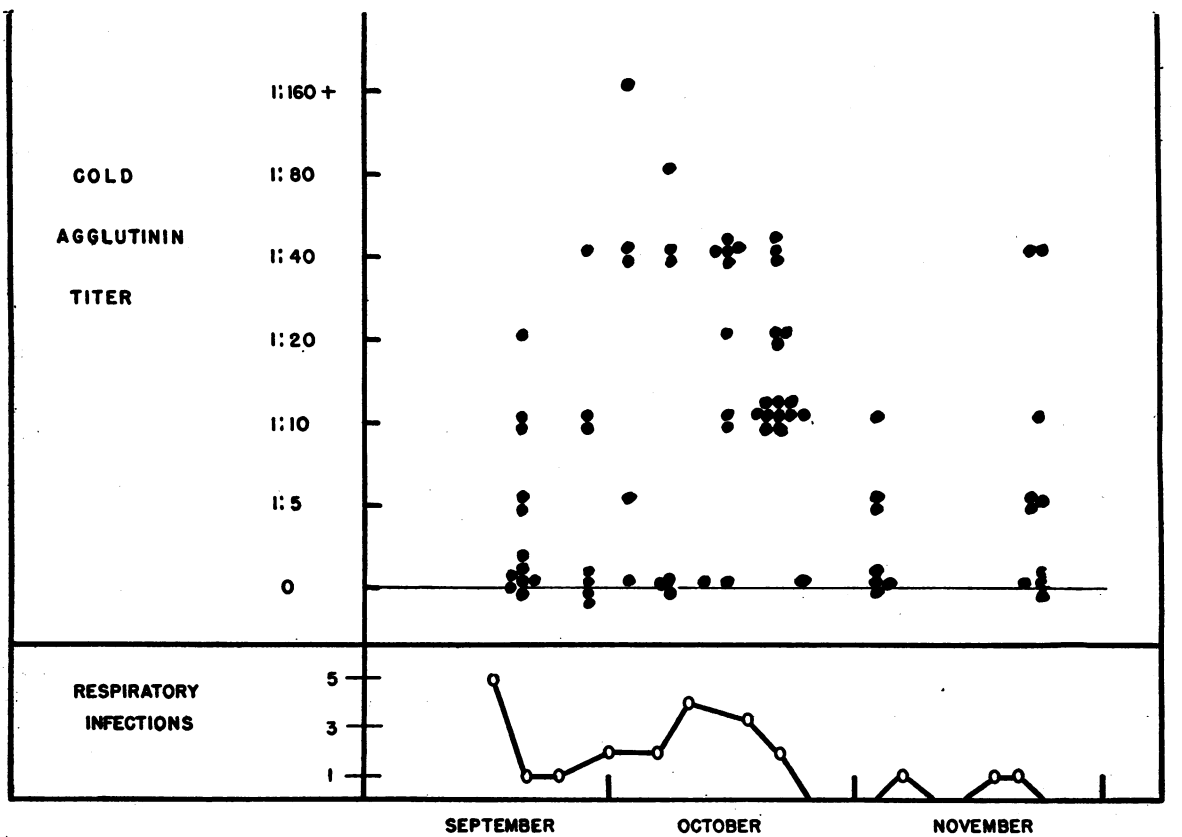

FIG. 1. A RECORD OF 73 COLD AGGLUTININ TESTS ON 27 NURSES COLLECTED FROM THE STUDENT NURSE GROUPS DISCUSSED IN THE TEXT

Ten had colds, 13 tracheobronchitis, and 1 cold agglutinins without symptoms. The number of respiratory infections indicates those whose onset was within the preceding 5 days. The increased number of infections correlates roughly with the elevated cold agglutinin titers.

TABLE I

Maximum cold agglutination titers *

Disease	0	1:5	1:10	1:20	1:40	1:80	1:160+	Total no. of patients
Atypical pneumonia	4	1	3	6	6	8	18	46
Tracheobronchitis	7	0	6	2	4	7	1	27
"Colds"	3	2	4	0	3	1	1	14
Influenza (complement fixation test positive)†	3	0	1	2	2	1	0	9

Summary: Atypical pneumonia—32 out of 46 patients had cold agglutinin titers of 1:40 or more (69 per cent); 18 of 46 patients had titers of 1:160 or more (39 per cent).

Tracheobronchitis—12 out of 27 patients had cold agglutinin titers of 1:40 or more (45 per cent).

"Colds"—5 out of 15 patients had cold agglutinin titers of 1:40 or more.

Influenza—9 patients with clinical influenza occurring during a recognized epidemic had influenza A complement fixing antibodies rising during the course to 1:64 or higher. Two patients who had cold agglutinin titers during influenza had had a previous attack of tracheobronchitis also accompanied by similar cold agglutinin titers. Neither of the 2 had cold agglutinin titers during the interval before developing influenza.

* Each patient had 2 or more sera tested, one of them 14 days or later after the acute onset of his disease.

† The author wishes to acknowledge the kind assistance of Dr. John F. Enders of the Harvard Medical School in whose laboratory the complement fixation tests were performed.

has maintained a titer of 1:40 during the winter (4 months).

In October of 1943, 22 second and third-year student nurses, also reporting for their physical check-ups and chest x-rays, were studied in the same fashion as the above group. Six of these had cold agglutinin titers of 1:20 or higher (included in Table I). A careful history showed that all but one of these with cold agglutinins had had a "cold" or tracheobronchitis within the previous 2 weeks. The one exception was admitted to the hospital 2 days later with a classical tuberculous pleurisy with effusion. This student nurse has since been followed and found to have a titer rising to 1:40 during the height of symptoms and a return to normal in convalescence. In the others with respiratory infections, the titers have steadily returned toward negative.

Hereditary cold agglutinins have been postulated (7). In Table II are records on 3 patients who have maintained high cold agglutinin titers for long periods of time despite minimal stimuli. If one of these patients had been tested for cold agglutinins between infections, he might well have been considered an example of hereditary cold agglutinemia.

TABLE II

Cases of recurring cold agglutinins

	Date	Titer	Day of disease
Miss M. E.			
Atypical pneumonia	4/20/43		
German measles	5/15/43		
	5/25/43	1:40	10th
	6/24/43	1:40	40th
	7/20/43	1:40	66th
Begin daily exposure, atypical pneumonia	8/25/43		
Tracheobronchitis, mild	9/3/43		
	9/17/43	1:160	14th
	9/21/43	1:80	18th
	9/28/43	1:80	25th
Very slight cough	10/6/43		
Malaise, temperature 101.4° 1 day	10/7/43	1:320	2nd
Slight cough	10/10/43	1:40	5th
Well	10/28/43	1:40	23rd
Slight cough	11/3/43	1:80	29th
	11/15/43	1:20	41st
Gastro-intestinal upset, temperature 100.4° 1 day	11/18/43		
	11/22/43	1:80	48th
Well	12/6/43	1:80	61st
Chronic cough 1 month			
Heavy exposure to atypical pneumonia December to present	2/16/44	1:160	30th
Dr. R. B.			
Atypical pneumonia, mild	6/23/43	1:20	7th
	6/26/43	1:40	10th
Well	7/1/43	1:40	15th
	7/7/43	1:40	21st
	7/10/43	1:20	24th
Tracheobronchitis, mild	9/20/43		
	10/4/43	1:80	14th
Still slight cough	10/15/43	1:20	25th
Very slight cough	11/10/43	1:5	51st
Very slight cough	12/31/43	1:10	102nd
Miss J. C.			
Onset, atypical pneumonia	5/2/43		
	5/4/43		2nd
	5/6/43	1:10	4th
	5/9/43	1:40	7th
Afebrile	5/12/43	1:40	10th
	5/15/43	1:40	13th
	5/18/43	1:80	16th
Cough gone, patient well	6/7/43	1:40	36th
	7/1/43	1:40	60th
	7/26/43	1:20	85th
Begin heavy exposure, atypical pneumonia	8/14/43		
	9/22/43	1:40	39th
	9/29/43	1:160	46th
	11/3/43	1:40	81st
	11/15/43	1:80	93rd
Slight "flu"; ambulatory	12/1/43		
	12/3/43	1:80	111th
	12/14/43	1:80	122nd
	1/12/44	1:20	151st
Roommate "cold" 4 days before	2/16/44	1:160	183rd

TABLE III

Cold agglutinins in other diseases

Disease	Comment	Number of patients	Maximum titer
	Virus diseases		
Rubella	1 month after atypical pneumonia	1	1:80
	1 month after scarlet fever	1	1:40
	Mild	1	1:20
	Mild	1	1:10
		1	1:5
		2	
		7	
Measles	With bronchopneumonia	1	1:40
	Severe	1	1:40
	Mild	2	
		4	
Chicken pox	Mild	1	1:40
	Mild	1	1:5
		2	
Mumps		1	
	With meningitis	1	1:20
	With meningitis	1	1:5
	Moderately severe	1	1:40
		4	
Ornithosis		1	1:80
		2	
		3	
Trachoma		1	1:80
Trachoma		1	1:40
		2	
Lymphopathia venereum		1	1:10
Lymphopathia venereum		1	
		2	
Meningo-encephalitis		2	
	Bacterial diseases		
Beta hemolytic streptococcus	Pharyngitis	1	1:80
	Pharyngitis	2	1:5
	Pharyngitis	3	
	Otitis media chronic	1	1:80
	Severe surgical scarlet fever	1	
	Severe scarlet fever	1	1:80
	Severe scarlet fever	1	1:20
		1	1:20
	Moderate scarlet fever	1	1:10
	Moderate scarlet fever	3	1:5
		7	
		22	

TABLE III—(*Continued*)

Disease	Comment	Number of patients	Maximum titer
	Bacterial diseases—(Continued)		
Pneumonia	Bronchopneumonia	1	1:5
	Bronchopneumonia	5	
	Severe type II; empyema; serum treatment	1	1:10
	Pneumonia with empyema	1	1:40
	Pneumonia with lung abscess	1	1:160
	Pneumonia with lung abscess and empyema	1	1:40
	Type IV and ? atypical pneumonia	1	1:10
	Classical lobar	1	1:20
	Classical lobar	6	
		18	
Tuberculosis	Childhood type simulating atypical pneumonia	1	1:5
	Epituberculosis	1	
	Pleurisy with effusion	1	1:40
	Pleurisy with effusion	1	1:20
		1	1:10
		1	
	Pulmonary with superimposed broncho-pneumonia	1	
	Minimal apical	1	1:5
	Minimal apical	1	
	Miliary	1	
	Widespread pulmonary	1	1:10
	Widespread pulmonary	1	1:10
		1	1:5
		1	
		14	
Endocarditis	Subacute bacterial endocarditis, para-influenza	1	1:40
	Subacute bacterial endocarditis, streptococcus viridans	2	
	Acute staphylococcus aureus	1	
		4	
	Blood diseases		
Acute lymphatic leukemia		1	1:40
Acute lymphatic leukemia		1	
Chronic hemolytic anemia with jaundice		1	1:160
Chronic myeloid leukemia		3	
Terminal myeloid leukemia		1	1:280,000
Polycythemia-leukemia		1	
Untreated severe pernicious anemia		1	

TABLE III—(*Continued*)

Disease	Comment	Number of patients	Maximum titer
Blood diseases—(Continued)			
Infectious mononucleosis		2	1:80
		1	1:20
	With jaundice	1	1:40
	With glands and sore throat	2	1:20
	With glands and sore throat	4	
		10	
Miscellaneous diseases			
Asthmatic bronchitis		1	1:20
		1	1:10
		2	1:5
		4	
Acute rheumatic fever			
	Rheumatic fever with chorea	1	
	Exacerbation, chronic	1	
	Acute rheumatic fever	5	
		7	
Pulmonary edema		1	1:5
Pulmonary edema		1	
		2	
Chronic bronchiectasis and pulmonary fibrosis		1	
Extensive metastasis to lung		2	
Carcinoma bronchus, advanced		2	
Acute hepatitis		1	1:40
Acute hepatitis		1	1:80
		2	
Mild hepatitis with jaundice		1	1:20
Mild hepatitis with jaundice		1	1:10
		1	1:5
		1	
		4	
Catarrhal jaundice		1	1:5
Catarrhal jaundice		2	
		3	
Terminal cirrhosis		1	
Hepatitis without jaundice		1	
Ulcerative colitis		1	
Syphilis		1	1:5
Syphilis		20	
		21	

TABLE III—(*Continued*)

Disease	Comment	Number of patients	Maximum titer
Miscellaneous diseases—(Continued)			
Chancroid		1	1:10
Disseminated lupus erythematosus		2	1:80
		2	
		4	
Sarcoid		1	
Scleroderma		1	1:5
	With esophageal stricture	1	1:20
		2	
Acute focal myocarditis		1	1:10
Mikulicz syndrome		1	
Multiple myeloma		1	
Bronchial asthma		1	1:10
Bronchial asthma		2	
		3	
Erythema nodosum		1	1:20
Erythema nodosum		4	
		5	
Insulin allergy		1	1:40
Following massive intravenous typhoid for fever therapy		1	
Severe ivy poisoning		1	
Allergic rash		1	

DISCUSSION

Why cold agglutinins should be frequent among patients in a general hospital, as is indicated in the tables, is not clear. A sensitive method and the listing of only the maximum titer chosen from multiple tests on each subject are contributing reasons. Higher readings also may have resulted from careful collection of serum without chilling blood below room temperature before storing serum (3). Multiple tests on a single person have also demonstrated the persistence of cold agglutinins for months following illness, seasonal changes of titer with or without accompanying common respiratory infections, and rises in titer with different infections. Whether these ex-

amples represent an endemic disease due to a specific agent, an institutional infection, or a chronic individual infection is not known. That low titers occur with minor respiratory infections, however, is known (2). Although cold agglutinins have not been found with lobar pneumonia and febrile conditions treated with sulfonamides (1) nor in normal subjects (3), controls taken from the variety of patients here presented have not been reported.

In the present patients, serum cold agglutination titers up to 1:40 were found in association with a number of acute infectious diseases due to viruses and bacteria, as well as associated with many miscellaneous conditions, including hepatitis, leukemia, and idiopathic hemolytic anemia. Similarly, a great many normal subjects were found to have cold agglutinin titers within the same range. These normals, however, usually had a history of or showed signs of an infection antedating the presence of cold agglutinins in their sera. Furthermore, these normals usually lost their cold agglutinins with the passage of time or later developed an infection in turn followed by a further serum cold agglutinin response.

In the present study, serum cold agglutinin titers of 1:160 or more were uncommon. Atypical pneumonia, the common cold, severe lobar pneumonia with empyema, chronic idiopathic hemolytic anemia, and myeloid leukemia were the only conditions in which so high a titer was present. Eighteen out of 46 atypical pneumonia patients had a serum cold agglutinin titer of 1:160 or higher.

In our group, a cold agglutinin titer of 1:40 or more was present in many conditions. Thirty-two out of 46 atypical pneumonia patients, 12 out of 27 tracheobronchitis patients, 5 out of 14 patients with "colds", and scattered patients with rubella, measles, chicken pox, mumps, ornithosis, trachoma, streptococcal infections, pneumonia, tuberculosis, endocarditis, hepatitis, allergy, leukemia, and infectious mononucleosis, had cold agglutinin titers of 1:40 to 1:80. From these findings, it would seem that the significance of serum cold agglutinins depends not only on the clinical findings in the patient but also upon the amount of agglutination. Although cold agglutinins were present in atypical pneumonia serum more often than in the other diseases studied in this report, only titers of 1:160 or more were well above titers observed in the variety of acute and chronic conditions seen in a general hospital. Usually, atypical pneumonia patients with this high cold agglutinin titer had the more severe illnesses in which the diagnosis was well established on clinical grounds.

SUMMARY

1. The present report confirms and extends the list of conditions associated with the presence of cold agglutinins.

2. Cold agglutinins may be associated with exposure to a variety of noxious stimuli: Drugs, bacterial toxins, parasites, viruses, and blood letting. Common coughs and colds may be associated with cold agglutinins.

3. Several individuals with high cold agglutinin titers (1:160+) following one infection have been found to have rises in titer with subsequent infections. Cold agglutinins may persist after atypical pneumonia for 9 months, or cold agglutinins may appear and disappear without evident infection.

4. Atypical pneumonia is more often associated with the presence of serum cold agglutinins than other diseases studied in this report. A titer of 1:40 may be present in a variety of diseases. Among hospital patients in Boston, a titer of 1:160 is uncommon except in severe atypical pneumonia. When interpreted with other findings, the cold agglutinin test is useful in clinical diagnosis.

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