STUDIES OF HUMAN ANTI-γ-GLOBULIN FACTORS REACTING WITH PEPSIN-DIGESTED γ-GLOBULINS*

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Relatively buried antigenic determinants of γ-globulin revealed by pepsin digestion which react with human anti-γ-globulin factors were first described by Osterland, Harboe, and Kunkel (1). Subsequent observations (2, 3) have emphasized that these anti-γ-globulin factors showing specificity for the pepsin site are often associated with sera containing so-called "anti-antibodies" (4–6). During a study of human sera containing anti-γ-globulin factors showing specificity for sites revealed on γ-globulin by pepsin digestion, evidence for marked autospecificity was obtained. Although no prozones have been noted in several reports concerning this class of anti-γ-globulin factors (1–3, 7), studies outlined below produced evidence that patients possessing high titers of antibody reacting with cells coated with pepsin-digested anti-CD Ripley had inhibitors in their sera which were both auto- and isospecific. The possiblity was entertained, therefore, that something possessing determinants of the 5S pepsin fragment of γ-globulin (8) existed free in serum of such patients.[1] Further studies reported here provide evidence that sera from normal individuals or patients with a variety of disorders contain low molecular weight 3S–5S fractions of slow gamma mobility capable of inhibiting agglutination reactions directed at the pepsin site. Evidence is presented that such lower molecular weight γ-globulins possessing antigenic determinants present in pepsin-digested γ-globulin are present in native human serum. The coexistence in normal human sera of antibody to sites on γ-globulin exposed by pepsin digestion and low molecular weight γ-globulin components possessing antigenic features present on the pepsin site provides an example of naturally occurring autoreactivity.

Materials and Methods

Human sera containing anti-γ-globulin factors with specificity for the pepsin site were found by screening 400 sera from hospital in-patients and out-patients. Rh-positive (R_1R_1)

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[1]The 5S pepsin fragment of γ-globulin will be designated $F(ab')_2$; the 3.5S S fragment, Fab; and the portion of the S fragment comprised by heavy chains, Fd according to the terminology suggested by the World Health Organization (8).

cells coated with pepsin-digested incomplete anti-Rh antibody Ripley (1–3, 7) were used in a slide agglutination technique as previously described. A group of seven sera with particularly high agglutinating titers for cells coated with pepsin-digested anti-Rh antibody Ripley was selected for study. Titers ranged from 1:256 to 1:2048 (Table I).

Serum fractions were isolated by zone electrophoresis in starch (9) using 0.1 ionic strength Veronal buffer, pH 8.6; by Sephadex G-200 gel filtration in 0.15 M NaCl; by sucrose gradient ultracentrifugation for 16 hr at 35,000 rpm using gradients made 5–20%, 5–30%, or 10–40% in sucrose (10); and by DEAE Sephadex chromatography using stepwise elution with 0.02 M pH 8.0 phosphate, 0.15 M pH 6.8 phosphate, and 0.33 M pH 4.2 phosphate buffers.

Inhibition tests were done as previously described (7) using a dose of agglutinating serum consistently showing a 2+ agglutination. Inhibitors were adjusted to 1 mg/cc protein concentration and tested in doubling dilutions, using as agglutinators four different sera V. G., L. B., D. K., and M. S.

5S pepsin fragment of γG was labeled with ^{125}I, two atoms per mole protein, by the method of Helmkamp (11). The labeled protein was cleared of aggregates by ultracentrifugation at 25,000 rpm for 2 hr, and added to various test sera.

Isolated γ-globulin preparations and sera from the patients with high titers of agglutinating antibody for the pepsin site were examined in the Model E ultracentrifuge using techniques previously described (12, 13).

Attempts at separation of smaller γ-globulin components from the bulk of 7S γG employed Sephadex G-200 gel filtration at pH 4.0, using acetate buffers of 0.1 ionic strength. γ-Globulin fractions from zone electrophoretic separations were equilibrated with 0.1 M acetate buffer pH 4.0 by dialysis and applied to Sephadex G-200 columns. Fractions were tested in immune diffusion against rabbit antisera specific for human Fc fragment, $F(ab')_2$, and against anti-$F(ab')_2$ antiserum absorbed with pooled L chains from Cohn fraction II. (7)

RESULTS

The studies contained in this report were prompted by an unexpected finding obtained with γ-globulin fractions from the first peak of a DEAE-cellulose column separation using the highest titered serum (L.B., Table I). Because sucrose density gradient ultracentrifugation and Sephadex G-200 gel filtration had confirmed the predominant 7S distribution of agglutinating activity in most sera studied, serum L. B. was equilibrated with pH 8.0, 0.02 M phosphate buffer and applied to a DEAE-cellulose column similarly equilibrated. The first fall-through peak with starting buffer contained agglutinating activity as shown in Fig. 1. However, when this peak was concentrated by ultrafiltration through collodion membranes in the cold at 4°C, the residual concentrate contained no aggluntinating activity for cells coated with pepsin-digested human incomplete antibody Ripley. The same results were found in the case of the six other high-titered sera applied to DEAE-cellulose columns. Ultracentrifugal examinations of the concentrated fall-through peaks from DEAE-cellulose in all instances showed symmetrical 7S peaks. This apparent disappearance of strong serologic activity suggested the presence of autologous inhibiting substances.

Because serologic activity had mysteriously disappeared in starting buffer DEAE-cellulose preparations from all high-titered sera, inhibition tests were performed using dilutions of native serum agglutinator and material from con-

centrated, pooled fall-through peaks of DEAE-cellulose columns equilibrated with pH 8.0, 0.02 M phosphate buffers as inhibitor. The results are shown in Table II. It can be seen that in all instances, inhibitory capacity showing both iso- and autospecificity was noted in these preparations. Pepsin digestion of autologous individual γ-globulins isolated by zone electrophoresis produced inhibition to $\log_2$ dilution of 12. On the basis of this and previous work utilizing

TABLE I

Summary of Clinical and Serological Findings among Seven Patients Showing Unusually High Titers of Agglutinating Antibody for Cells Coated with Pepsin-Digested Anti-CD Antibody Ripley

Patient (sex age)	Diagnosis	Gm type of whole serum	Titer with cells coated with pepsin-digested anti-CD Ripley*	Molecular distribution of pepsin site agglutinator by sucrose gradient ultracentrifugation and/or Sephadex G-200 gel filtration
L. B. (F-62)	Hamman-Rich syndrome	Gm (1, 4, 5)	2048	7S
M. S. (F-76)	Metastatic carcinoma	Gm (1, 4, 5)	1024	7S, 19S
D. K. (M-64)	Exfoliative dermatitis	Gm (−1, 4, 5)	512	7S
P. S. (M-77)	Chronic lymphatic leukemia	Gm (−1, 4, 5)	256	7S, 19S
V. G. (F-39)	Rheumatoid arthritis	Gm (1, −4, −5)	256	7S
J. W. (M-75)	Carcinoma of stomach	Gm (1, 4, 5)	256	7S
V. F. (F-45)	Rheumatoid arthritis	Gm (−1, 4, 5)	256	7S

* Whole undigested anti-CD Ripley Gm (1, 4, 5).

pepsin-digested myeloma proteins (7), it was possible to estimate that concentrations of 0.03–0.015 mg/cc of material bearing antigenic determinants similar to those exposed by pepsin digestion of γG were present in the fractions from DEAE-cellulose columns tested.

In addition, initial peaks obtained from three normal sera by DEAE-cellulose columns at pH 8.0, 0.02 M phosphate were tested for inhibitory capacity. In all instances, these fractions displayed distinct inhibitory capacity of $\log_2$ dilution 2-4 for several agglutinators and cells coated with pepsin-digested anti-CD Ripley. Thus, inhibitor could be obtained from normal sera negative in the whole state for antibody activity directed at the pepsin site.

Since concentration of DEAE-cellulose column peaks had produced loss or

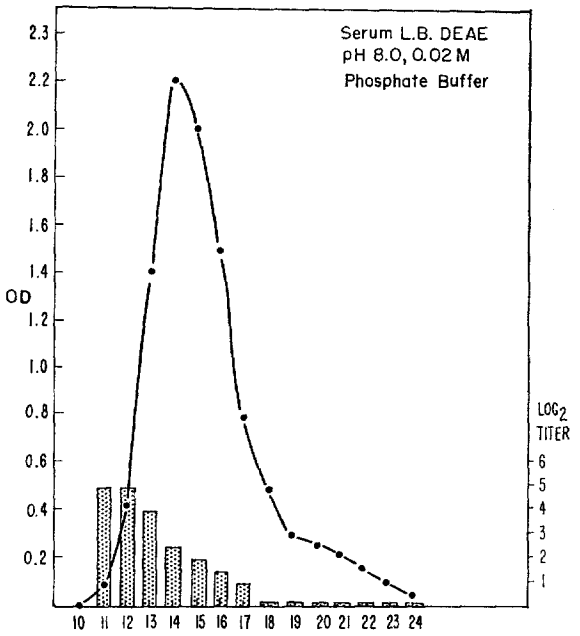

FIG. 1. Protein curve and agglutinating activity for cells coated with pepsin-digested anti-CD Ripley in serum L. B. applied to DEAE-cellulose column equilibrated with pH 8.0, 0.02 M phosphate buffer. When fractions 11–19 were concentrated in the cold by pressure through collodion membranes, all agglutinating activity for pepsin-digested γG disappeared. Essentially the same result was noted with six other high titered sera.

TABLE II

Inhibition of Agglutination of Six Whole Agglutinating Sera Reacting with Cells Coated by Pepsin-Digested Anti-CD Ripley by Initial Peaks from DEAE Column Separations

Agglutinator adjusted to give 2–3+ agglutination	Inhibitors tested				
	L.B., 1st DEAE peak*	M.S., 1st DEAE peak	V.G., 1st DEAE peak	P.S. 1st DEAE peak	5S pepsin digest‡
L. B.	6§	5–6	6	4	L. B., 5S pepsin digest 12
M. S.	6	6	6	1–2	M. S., 5S pepsin digest 12
D. K.	6	5–6	6	4	D. K., 5S pepsin digest 12
J. W.	6	6	6	3–4	J. W., 5S pepsin digest 12
P. S.	6	6	6	8	P. S., 5S pepsin digest 12
V. G.	6	6	6	6	V. G., 5S pepsin digest 12

* Refers to first peak obtained when serum applied to DEAE column pH 8.0, 0.02 M phosphate buffer.

‡ 5S pepsin fragment obtained from pepsin digestion of isolated individual γG.

§ Log_2 dilution of inhibitor effective in inhibiting agglutination. Inhibitors were adjusted to 1 mg/cc.

abolition of serologic agglutinating activity for the pepsin site, it seemed possible that some cleavage had occurred spontaneously within the various γ-globulin fractions. That such splitting might occur after storage of isolated myeloma proteins for 1–3 wk was apparent in an earlier study (7). In an attempt to study this problem further, fresh sera collected from normal blood donors were tested within 12 hr of separation from the cells by centrifugation. These sera were not subjected to any fractionation procedures, but were tested in the neat and diluted state for inhibitory capacity for the pepsin site. 5 of the 10 sera so tested showed concordant inhibition of three different agglutinators. Two other sera inhibited two of the three agglutinators. Thus, in 7 of the 10 fresh sera tested inhibitory activity for the pepsin site was found.

Separation Procedure Used in an Attempt to Characterize Autologous Inhibitors.—The next procedure of separation applied was zone electrophoresis (9). The distribution of γG, γA, and γM, as well as the results of agglutination and inhibition reactions are depicted in Fig. 2. It can be seen that agglutinating activity for cells coated with pepsin-digested anti-CD Ripley was detected in γ-, β-, and albumin zones of sera which showed agglutinating activity in the whole state. In addition, normal sera devoid of agglutinating activity in the whole state showed weak agglutination in the albumin region. The reaction in the albumin region of both negative and positive sera was not inhibited by γG, 5S pepsin fragment, 3.5S S fragment, or normal serum albumin. Control cells (including CD negative cells), not coated with pepsin-digested anti-CD Ripley, were agglutinated to the same degree as coated R_1R_1 cells, and activity toward coated cells was abolished by absorption of the appropriate fractions with washed uncoated cells. The nature of this agglutinating activity was not investigated further, but resembles the albumin-active, autoagglutinating system reported by others (14).

Of special interest was the finding that distinct inhibition could be found in some of the slow starch block fractions devoid of agglutinating activity. Such fractions showed precipitins by immune diffusion in some instances with rabbit antisera to $F(ab')_2$, but not with anti-Fc. The most clearcut inhibition was notable in slow γ-globulin fractions from zone electrophoretic separations of normal serum not possessing agglutinating activity for pepsin-digested, anti-CD Ripley–coated cells in the whole unfractionated state (Fig. 2, normal serum V2-180). Thus, in positive and negative sera, fractions of slow γ-electrophoretic mobility or appearing in the fall-through peak of starting buffer for DEAE-cellulose columns appeared to contain antigenic determinants similar to those involved in the pepsin site antigen.

The next approach towards characterizing inhibiting substances in both positive and negative sera employed ultracentrifugation in sucrose gradients and Sephadex G-200 gel filtration. An example of detection of inhibiting capacity after sucrose gradient separation is shown in Fig. 3. Inhibition of several agglutinating systems specific for the pepsin site as manifested by cells coated

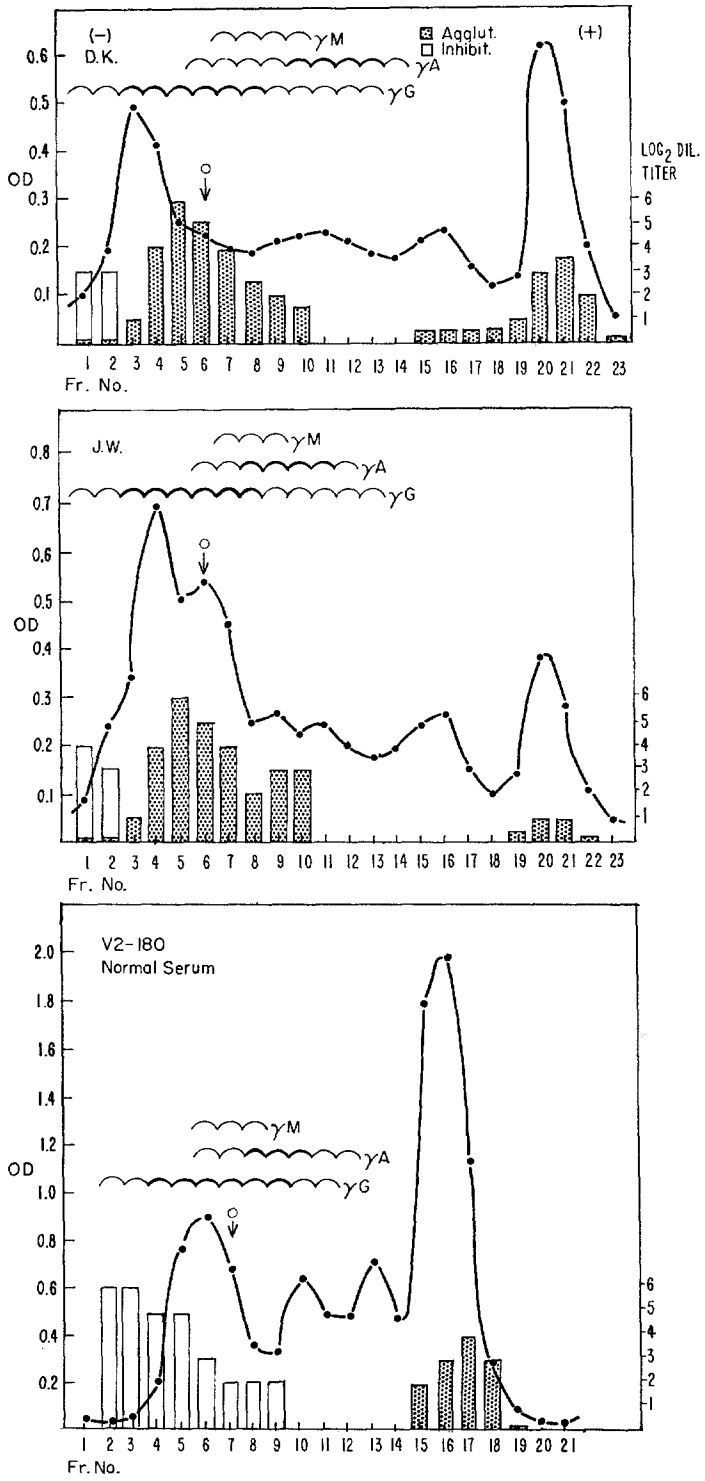

FIG. 2. Zone electrophoresis separations of two sera, D. K. and J. W., showing distribution of agglutinating activity (Agglut.) for cells coated with pepsin-digested anti-CD Ripley as well as fractions of slow γ-electrophoretic mobility which showed inhibitory capacity (Inhibit.) The normal serum V2-180 shown below revealed marked inhibitory capacity in slow γ-globulin fractions. Agglutinations noted in albumin zones were not inhibited by pepsin-digested γ-globulin nor by albumin and could be absorbed out with uncoated human cells. They were, therefore, felt to be a separate phenomenon and not studied further.

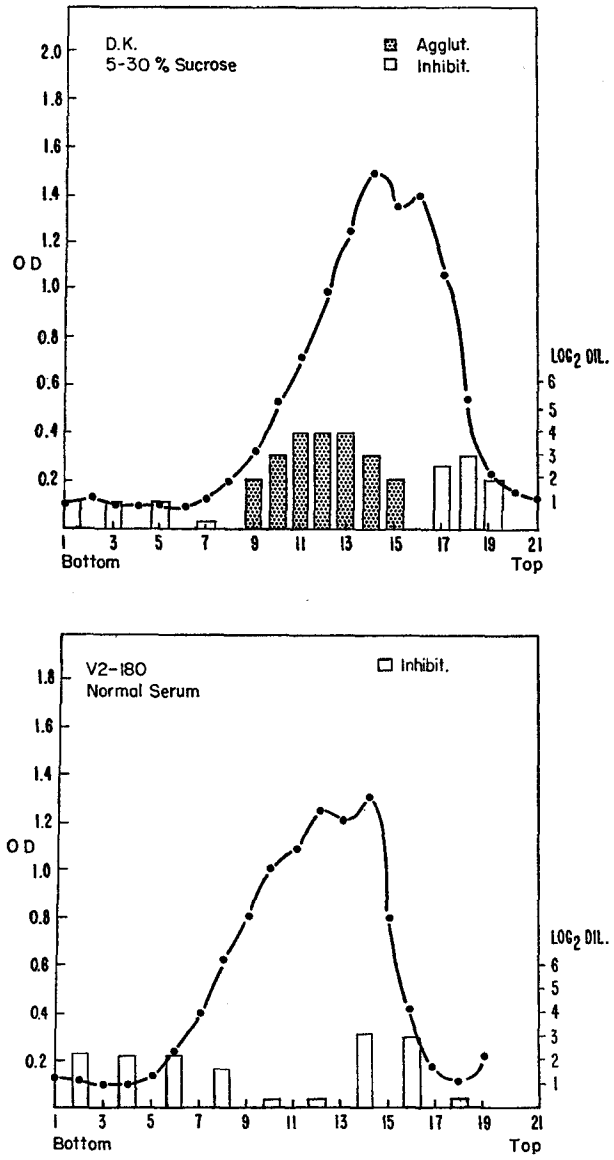

FIG. 3. 5–30% sucrose gradient separation of serum D. K. possessing agglutinating activity (Agglut.) for cells coated with pepsin-digested anti-CD Ripley predominantly in the 7S region. Inhibition (Inhibit.) was notable in 3S region as well as slight inhibitory effect below 7S region. The normal serum V2-180 not possessing detectable agglutinator shows a similar distribution of inhibitor. Control experiments with 20 and 30% sucrose revealed that these concentrations inhibited the agglutination reaction to a $\log_2$ dilution of 2. No inhibition was noted with 10 or 5% sucrose.

with pepsin-digested anti-CD Ripley was notable in fractions sedimenting well above the 7S zone. However, slight inhibition was also present in lower fractions and since sucrose alone in concentrations of 20–30% produced slight inhibition of agglutination systems, attention was next directed to gel filtration experiments.

Sephadex G-200 gel filtration of several high titered sera (L. B., D. K., V. G.) revealed serologic agglutinating activity for pepsin-digested, anti-CD Ripley–coated cells only in 7S regions, whereas two sera separated by this method (M. S. and P. S.) showed 7S and 19S agglutinating activity. When gel filtration fractions of both positive and negative pepsin-agglutinator sera were studied for their inhibitory capacity, consistent inhibition was noted in the lowest molecular weight fractions (5S or below). Of considerable interest was the finding that normal serum V2-180 showed weak but definite agglutination in 19S fractions from the Sephadex G-200 separation shown in Fig. 4, whereas the whole serum had been negative when tested with pepsin-digested, incomplete antibody Ripley–coated cells. These findings indicated concomitant agglutinating activity of 7S or 19S distribution along with the presence of inhibitor 3S–5S in both strongly positive and negative sera.

An attempt was next made to obtain low molecular weight material from positive and negative sera by Sephadex G-200 gel filtrations in acid buffer at pH 4.0. Slow starch block fractions from sera such as V2-180 (Fig. 2) were equilibrated by dialysis against acetate buffer pH 4.0, 0.1 M, and applied to columns equilibrated with the same buffer. In addition 7S fractions from previous Sephadex G-200 column separations in 0.15 M saline were applied to columns equilibrated with the pH 4.0 buffer. The results of such attempts at isolation of inhibitor are shown in Fig. 5. It can be seen that low molecular weight inhibiting material appearing in Sephadex G-200 effluents in the 3S–5S region could be recovered using these acid buffer conditions. Indeed, evidence for autologous previously complexed inhibitor was obtained in experiments such as that shown in the upper portion of Fig. 5. Here the 7S region of a previous Sephadex G-200 separation of serum L.B. was recycled over Sephadex G-200 in pH 4.0 buffer. Low molecular weight materials capable of inhibiting agglutinator L.B. as well as other sera were thereby recovered.

Further evidence related to the antigenic character of low molecular weight γ-globulins present in both positive and negative pepsin site–agglutinating sera was obtained by inhibition of precipitation reactions in agar gel. It was noted that such lower molecular weight materials inhibited precipitation reactions apparently preferential for antigenic sites on 5S pepsin fragment but not those reacting with Fc. In these reactions antisera made against the 5S pepsin fragment of γ-globulin were absorbed with 3.5S papain S fragment, providing an antiserum still showing residual precipitins for 5S pepsin fragment (7).

Some insight into possible complexing of 7S pepsin site agglutinators with

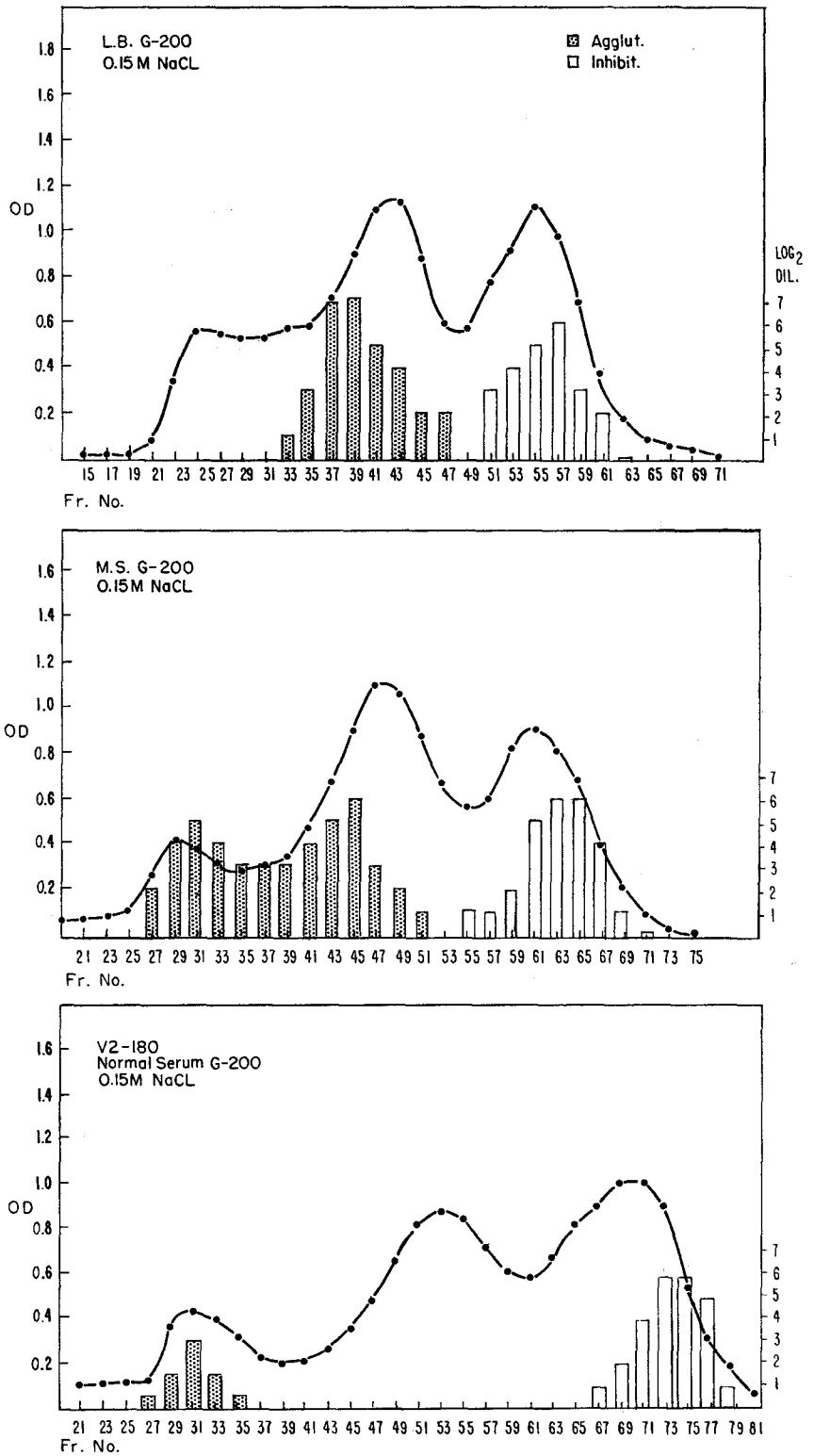

FIG. 4. Sephadex G-200 gel filtration separations of two sera, L. B. and M. S., showing 7S and 19S and 7S-agglutinating activity (Agglut.) for cells coated with pepsin-digested anti-CD Ripley. Distinct inhibition (Inhibit.) to $\log_2$ dilution of 5–6 was noted in lower molecular weight fractions 3S–5S.

The normal serum V2-180 shows weak 19S-agglutinating activity in separated state although it is negative when tested as whole serum. Of interest was concomitant inhibitor in serum V2-180 found in 3S region of Sephadex column separation. Low molecular weight 3S–5S inhibitor was similarly recovered from two other fresh normal sera by Sephadex G-200 filtration.

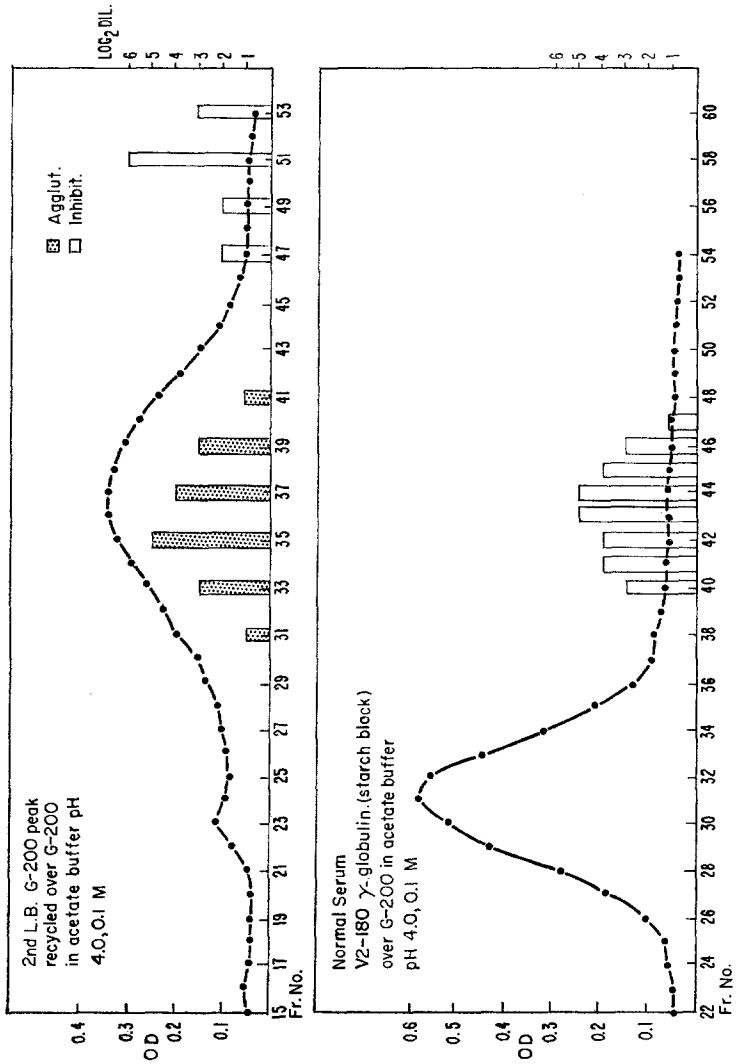

FIG. 5. Sephadex G-200 gel filtration in acid buffer pH 4.0. The second Sephadex G-200 peak from previous separation of serum L. B. was recycled (Agglut.) over an acid column and after dialysis back to pH 7.4 lower molecular weight inhibitors (Inhibit.) recovered.

Below is shown low molecular weight inhibitor (Inhibit.) obtained from slow starch block fractions of serum V2-180 after gel filtration at pH 4.0.

5S pepsin-digested γG-fragments was sought in two ways. 5S pepsin fragment labeled with ^{125}I was added to various sera both positive and negative in the whole state for agglutinating activity against pepsin-digested, anti-CD Ripley–coated cells. After incubation at room temperature for 2 hr, ultracentrifugation for 16 hr in 10–40% sucrose gradients failed to show consistent shifts in curves of radioactivity when labeled 5S pepsin γG-fragments had been added to sera possessing high titers of pepsin site agglutinators. In similar fashion, 5S pepsin fragments of γG when added to whole serum possessing high titers of agglutinating activity for pepsin-digested, anti-CD Ripley–coated cells showed no discernible new complexes in the analytical ultracentrifuge.

Similar experiments were performed using sera positive and negative for pepsin site–agglutinating activity and incubation at room temperature for 2 hr with ^{125}I-labeled 5S pepsin fragment of γG. Subsequent zone electrophoresis of serum-^{125}I $F(ab')_2$ mixtures in parallel with labeled 5S pepsin fragment alone showed no detectable shift in specific electrophoretic mobility of labeled material within sera showing high titers of pepsin site–agglutinating activity.

Quantitative precipitin curves were constructed using ^{125}I-labeled 5S pepsin fragment. Two sera with strong agglutinating activity toward Rh-positive cells coated with pepsin-digested anti-CD Ripley were selected. Using 0.2 cc of serum and from 0.2 to 86 μg of $F(ab')_2$ per tube no significant deviation from the antigen control could be detected by either protein determination or radioactive counting of the washed precipitates. Thus, no precipitating antibody for sites revealed on γG by pepsin digestion was apparent.

DISCUSSION

The studies described above were performed primarily because of the relatively high frequency in normal human sera of agglutinating antibodies with specificity for sites on γ-globulin uncovered by pepsin digestion. The initial reports describing this type of anti–γ-globulin factor (1) as well as subsequent surveys (7)[2] of a large number of sera from normal blood donors and patients in hospital have shown that about one third of all human sera contained this type of antibody. The incidence rises precipitously with age, and in sera from 60 patients between the ages of 60 and 80 yr pepsin site agglutinators were found in some 60%.[2] Since this variety of anti–γ-globulin factor is relatively common among normal human sera, some insight into its genesis and specificity seems warranted.

The clearcut results of autologous inhibitory substances were actually uncovered by isolation procedures when DEAE-cellulose peaks recovered with starting pH 8.0, 0.02 M phosphate buffers were concentrated and lost serologic activity. The most direct demonstration of native inhibitory materials which

[2] I. D. Wilson and R. C. Williams, Jr. Unpublished observations.

resembled or contained antigenic structures similar to those uncovered on pepsin-digested γG was notable in the zone electrophoresis separations of normal sera negative for pepsin site–agglutinating activity in the whole state. In all of eight such instances tested, inhibition was localized to the slow γ-globulin fractions of starch block separations. Moreover, a clear estimation of the relative size of inhibitory materials present in native serum was gained by inhibition experiments from Sephadex G-200 gel filtration separation procedures. In all instances, predominant inhibitory capacity was noted in the 3S–5S fractions. Some enrichment or relative increment of smaller γ–globulin or lower molecular weight–inhibiting material was gained when Sephadex G-200 separations were performed in an acid *millieux* at pH 4.0 designed to dissociate 7S antibody from 5S or small antigen. Indeed, acid conditions were not a requisite of the latter and separation of agglutinator from smaller inhibitor could be achieved using 0.15 M NaCl Sephadex G-200 column filtration. This suggests that if autologous antigen-antibody complexes exist in normal sera possessing high titers of pepsin site–agglutinating activity, their dissociation is relatively easy and perhaps a continuous dynamic phenomenon.

The possibility remains that spontaneous fracture of autologous native γ-globulins may occur and be responsible for the low molecular weight materials antigenically related to the 5S pepsin fragment and detected in this study. Fresh whole normal human sera tested within several hours of collection showed inhibitory capacity for pepsin site agglutination systems. It is conceivable that the clotting process itself may actuate some breakdown or alteration in γ-globulin fractions of serum. Pertinent to this entire problem are the recent reports of demonstrable proteolytic activity in various γ-globulin preparations (15, 16).[3] The lability or ease of exposure of the relatively buried antigenic determinant on pepsin-digested γ-globulin therefore becomes important. Studies by Waller[4] have recently indicated that complete digestion of the Fc or F fragment may not be necessary to expose the pepsin site antigen. The ease of exposure of this antigenic structure and the large proportion of normal human sera containing antibody directed towards it are of interest.

The exact nature of the 3S–5S autologous inhibitor present in sera with antibodies directed at the pepsin site is still not clear. The technique of inhibition of agglutination shown previously to be capable of detecting 5S pepsin-digested γ-globulin or pepsin fragments of various myeloma proteins in $\log_2$ dilution of 11-12 or 0.0008 mg/cc (7) was certainly much more sensitive than immune diffusion or agar gel precipitation methods used in general fashion to calibrate distribution of γG-antigenic determinants. That autologous inhibitor was not necessarily antigen-antibody complexes was clear from its distri-

[3]B. Robert and R. S. Blockman. 1966. Studies on the proteolytic activity of γ-preparations. Information Exchange Group No. 5. Scientific Memo No. 207.

[4]M. Waller. 1966. Personal communication.

bution in Sephadex G-200 gel filtration experiments. Harboe and coworkers (2) and recently Natvig (3) have emphasized the concurrent appearance of anti-γ-globulin factors showing specificity for pepsin-digested γG, as well as sites uncovered or juxtaposed by combination of antibody with antigen; so-called "anti-antibodies" (4, 5).

Recent reports (3, 17) have demonstrated some apparent selective specificity of pepsin site agglutinators for pepsin-digested anti-Rh incomplete antibodies seemingly dependent on the Gm type of the native anti-Rh serum. Such specificity was not readily apparent in previous studies (1, 7) but may be directly implicated in the studies of autologous inhibition presented here. It is of interest that the Gm types of the native sera studied here were of disparate types, but that all contained at least one Gm factor present in the whole anti-CD Ripley used after pepsin digestion to coat Rh-positive cells. Four high titered sera agglutinating pepsin-digested, anti-CD Ripley–coated cells were Gm (1, 4, 5). Three were Gm (−1, 4, 5), and one was Gm (1, −4, −5) (18). Further study using a broader spectrum of single pepsin digested anti-Rh antibodies is needed to clarify this point. Pertinent to this matter are the findings in Table II, where some variation in inhibitory capacity was notable using DEAE-cellulose–isolated γ-globulin fractions of serum P.S. Gm (−1, 4, 5). Marked autospecificity, $\log_2$ dilution of 8, was notable for inhibition of serum P.S., but deficiency in inhibition for serum M.S. Gm (1, 4, 5) was present. Since low molecular weight inhibitor was present in all positive agglutinating sera studied, it seems likely that the primary specificity of pepsin site agglutinators is directed toward autologous determinants.

Finally, recent studies on low molecular weight γ-globulins (19–21) are directly pertinent to our finding of native serum 3S–5S inhibitors of γ-mobility. The studies by Fireman and coworkers (20) as well as later extensive studies of 3S plasma γ-globulins by Ikenaka, Gitlin, and Schmid (21) revealed immunologic cross-reactions between 7S γG, S fragment, and the 3S γ-globulins isolated from plasma. Peptide maps as well as amino acid composition of these 3S γ-globulin serum components indicated distinct differences from B chain or Bence Jones proteins. These differences make it seem unlikely that the 3S γ-globulins studied by these workers were directly derived as metabolic products or fragments of γG. Study of a sample of the 3S γ-globulin kindly furnished by Dr. Schmid showed that this material did not inhibit pepsin site agglutination reactions in concentrations up to 2 mg/cc. Another report of 3.5S γ-globulin antibodies has recently appeared (22). These studies along with a number of previous reports provide evidence that γ-globulin components of low molecular weight may exist in serum (23, 24). Relatively elevated levels of free L chains have recently been described by several groups (25, 26) in the case of serum sickness and disseminated lupus erythematosus. Subsequent observations have extended this concept in the case of certain patients

with multiple myeloma (27). The concept of free antigen or presumptive potential antigen such as the pepsin fragment of γ-globulin being present or complexed in serum is somewhat new. A situation would then be obtained in which patients with high titers of antibody or high levels of pepsin site agglutinators may have formed antibodies to determinants of γG revealed or uncovered during autologous immunoglobulin catabolism. If antibodies showing specificity for 5S pepsin digested γ-globulin or its Fd determinants (7, 17) are present in a large proportion of normal random human sera and if the initiating antigen be autologous γ-globulin antigenic structures present as a by-product of γ-globulin catabolism, then a physiogenic or normal autoantibody does not seem particularly disquieting. Further investigation into the physiologic function of antibodies showing pepsin site specificity is needed. Whether such factors are operative in monitoring catabolic rates of γG-globulin would be of interest. This is particularly pertinent in view of the rapid turnover and elimination of 5S pepsin fragment previously reported (28, 29). However, more recent studies by this same group (30) have indicated that complexes of specific Fab and antigen or $F(ab')_2$ and antigen (BSA) persist longer in the circulating state than Fab or $F(ab')_2$ alone. These latter observations are of particular importance to some of the findings in the currently reported study.

SUMMARY

Many sera from normal individuals as well as patients with various disease states contain agglutinating antibodies which show specificity for antigenic determinants of γ-globulin revealed by pepsin digestion at pH 4.1. Sera containing such agglutinating activity as well as sera negative for these agglutinators contain low molecular weight (3S–5S) components of slow γ-mobility which inhibit these agglutination reactions. Low molecular weight inhibitors show both auto- and isospecificity, and are antigenically related to the 5S pepsin fragment of γ-globulin. A common situation is thereby revealed in which human anti-γ-globulin antibodies showing specificity for pepsin-digested γ-globulins are present in serum along with low molecular weight γ-globulin components capable of inhibition. Autoreactivity or autospecificity of such anti-γ-globulin factors is a phenomenon shared by both normal human sera and sera from patients with various disease states.

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