Immunology, 1969, **17**, 897.

A Human Serum Immunoglobulin with Specificity for Certain Homologous Target Cells, which Induces Target Cell Damage by Normal Human Lymphocytes

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(*Received* 19*th March* 1969)

Summary. A factor has been found in a number of human sera which renders a polyploid strain of human liver cells, Chang cells, susceptible to damage by non-immune human lymphocytes. Sera possessing this factor are referred to as Factor Containing Sera (FCS). Such damage is assessed quantitatively by release of radioactive chromium from target cells. This factor has the chemical properties of IgG and can be absorbed out on Chang cells. Its specificity has been shown to be for Chang cells and not for human lymphocytes. Other homologous and heterologous target cells tested were not affected by this factor. The factor has not been shown to have any effect on Chang cell viability by itself, even in the presence of complement. Factors which inhibit target cell damage are shown to coexist with the factor which induces non-immune lymphocyte damage of Chang cells. The possible origin of this factor is discussed as is the role in immune reactions of target cell specific antibody which renders such cells susceptible to damage by non-immune lymphocytes.

INTRODUCTION

Allergic destruction of target cells *in vitro* can be accomplished by antibody, usually in the presence of complement, or by immune cells. In addition, target cell specific antibody may induce both increased phagocytosis of target cells by macrophages (Bennett, Old and Boyse, 1963) and target cell damage by lymphocytes from unimmunized donors (Moller, 1965). Subsequently, many authors have described damage to target cells, by non-immune lymphocytes in the presence of target cell specific antibody (Perlmann, Perlmann and Holm, 1968; Granger and Kolb, 1968; MacLennan and Loewi, 1968b).

Non-immune lymphocytes can also cause damage to target cells in the presence of phytohaemagglutinin (Holm, Perlmann and Weiner, 1964). Furthermore, lymphocytes which have been stimulated by antigen to which they are sensitive and which is unrelated

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to the target cell may cause increased target cell damage (Holm and Perlmann, 1967b). Damage to target cells by non-immune lymphocytes occurs rapidly, being first detectable within 1–3 hours of addition of lymphocytes to target cells (Holm and Perlmann, 1967a).

A rapid onset of lymphocyte damage excludes the possibility that this is due to a primary immune response such as that seen in the mixed lymphocyte reaction (Bain, Vas and Lowenstein, 1964; Holm and Perlmann, 1967b). Although lymphocyte division often occurs in situations where lymphocyte mediated cell damage occurs, neither X-irradiation (Moller, 1967) nor Actinomycin-D (Holm, 1967) in doses which effectively abolish lymphocyte division destroy the ability of lymphocytes to mediate target cell damage. Only viable lymphocytes, however, are capable of mediating target cell damage (Holm, 1967).

In this investigation we present results suggesting that an IgG fraction from certain human sera is capable of inducing damage by non-immune human lymphocytes towards a polyploid strain of homologous target cells (Chang cells), in a manner perhaps analogous to that described by Moller and other authors in animal experiments. The discovery of this factor arose from an investigation designed to see if target cell specific antibody could account for the finding of Hedberg (1967) that joint fluid cells from certain patients with chronic inflammatory joint disease destroy homologous target cells. He failed to demonstrate such an effect by peripheral blood lymphocytes from these patients. In his experiments diploid human foetal cells of both renal and cutaneous origin were used. We have obtained similar results using Chang cells as targets. No positive correlation between this phenomenon and the factor mentioned above has been demonstrated and this paper mainly describes some biological and chemical properties of that factor.

MATERIALS AND METHODS

Joint fluid

Joint fluid was collected in sterile containers, centrifuged at 400 ***g*** for 30 minutes and the supernatant filtered through a 0·45 μ Millipore filter.

Joint fluid cells

Cells were obtained from heparinized fluid (2 units/ml) treated with hyaluronidase (Fison's) so as to give a final concentration of 20 viscosity reducing units/ml. The fluid was incubated at 37° for 20 minutes before filtration through a 20-ml syringe loosely packed with sterile gauze. The filtrate was spun at 300 ***g*** for 10 minutes and the button was suspended in Parker 199 (Glaxo), with penicillin and streptomycin, enriched with 10 per cent heat-inactivated foetal bovine serum (Flow Laboratories batch L40023)(PFB 10). The cells were washed twice more in PFB 10 before counting in a haemocytometer under phase contrast. They were then diluted to $2{\cdot}5\times 10^6$ joint mononuclear cells/ml.

Peripheral blood lymphocytes

Venous blood was defibrinated with 4-mm glass beads before sedimentation with an equal volume of 3 per cent gelatin (Coulson and Chalmers, 1964). The cells were washed in PFB 10 before enumeration and suspension in PBF 10 at $2{\cdot}5\times 10^6$ mononuclear cells/ml.

Rabbit anti-Chang serum

This was prepared by multiple intradermal injection of 10^7 Chang cells in Freund's

complete adjuvant. Three weeks later 10^7 Chang cells in 1 ml of saline was given by intravenous injection. Serum was collected 2 weeks after the second immunization. The serum was inactivated at 56° for 30 minutes.

Target cells

All target cells were obtained from Flow Laboratories (Irvine, Scotland) and were grown in 8-oz. medical flat bottles with Eagle's minimum essential medium for suspension cultures (Flow) with 10 per cent foetal bovine serum (FBS), 1 per cent non-essential amino acids (Flow), 1 per cent fresh isotonic glutamine, penicillin 200 units/ml and streptomycin 100 μg/ml. Single cell suspensions of polyploid strains were obtained by shaking the bottles at least once every 48 hours, removing the medium and suspended cells and replenishing with fresh medium. Single-cell suspensions of diploid fibroblasts were only obtained after treating monolayers with 0·25 per cent trypsin (Difco) in Dulbecco Ca-free buffer for 20 minutes and filtering the resulting suspension through loosely packed gauze.

Protein concentrations

These were calculated on the basis of extinction at 280 mμ in a 1-cm cell. Solutions of equal optical density were compared.

Separation of proteins on Sephadex G-150

A column of approximately 800 ml bed volume was used. The flow rate was upwards at 0·5 ml/min. Two millilitres of serum was applied to the column. The washing medium was 0·15 M phosphate buffered saline at pH 7·2. The column was sterilized by recycling 0·002 per cent chlorhexidine in buffer and this was rinsed out with sterile buffer for 2 days before application of serum. Five-millilitre fractions were collected. The extinction at 280 mμ in a 1-cm cell was determined for each fraction. The peaks were then pooled, concentrated where necessary *in vacuo* in 8/32 in. visking tubing at 4°, filtered through a 0·45-μ millipore membrane and stored at −20°.

Separation of sera on DE 52 cellulose

A batch technique based on that of Stanworth (1960) was employed. DEAE cellulose (Whatman DE 52) was equilibrated first against 0·2 M phosphate buffer, pH 7·3, at 4° and then against 0·01 M phosphate buffer, pH 7·3. The half-saturated ammonium sulphate precipitate from whole serum was dissolved in and then dialysed against 0·01 M phosphate buffer. Three millilitres of this dialysate, mixed with 10 ml of 0·01 M phosphate buffer was then mixed with 25 g wet weight of equilibrated DE 52 from which all excess fluid had been removed by filtration. This mixture was kept at 4° with stirring for 2 hours. After this the free fluid was removed by filtration, the cellulose was mixed twice with a further 20 ml of 0·01 M phosphate buffer and the free fluid was again filtered off. This procedure was repeated with 0·025 M, 0·05 M and 0·15 M phosphate buffer at pH 7·3. The eluates at each molarity were pooled, concentrated as above, made isotonic and filtered through a Millipore filter before storing at −20°.

Isoelectric fractionation of 0·01 M cuts of serum from DE 52 cellulose

Fractions of whole serum eluted from DE 52 with 0·01 M buffer (0·01 M cut) were also prepared for isoelectric focusing as above except that the eluate was treated with a further

small quantity of fresh DE 52, equilibrated against 0·01 M buffer. This mixture was then filtered and concentrated to approximately 3 ml. This was dialysed against 2 per cent ampholine solution of the pH range to be used. The isoelectric column, volume 110 ml, ampholines pH range 5–7, 7–9 and 7–10, and the 337-M power pack were obtained from L.K.B. The column was siliconized. The fractionation was carried out after Svensson (1961). A density gradient of sucrose and ampholines was set up in the column. The sample containing the 0·01 M cut in 2 per cent ampholines was substituted for the low density fractions (6, 7 and 8 in the L.K.B. method sheet). The total concentration of ampholines was 1 per cent made up of mixtures of ampholines pH 5–7, 7–9 and 7–10 to give the appropriate pH range. The anode was placed at the base using phosphoric acid and the cathode, which was placed at the top, was of ethylene diamine. The column was cooled by running water at 10°. The initial voltage was 300 V at 4 mA increasing by 51 hours to 500 V at 2 mA. The anode was then sealed off and the cathode solution siphoned off. The column was emptied at a flow rate of 1 ml/min. One-millilitre fractions were collected, pH was measured, and fractions were pooled in pH ranges of one or fractions of one pH unit. These fractions were dialysed to remove sucrose and ampholines and concentrated to equal protein concentrations for assay.

Cytotoxicity test procedure

Target cell damage was assessed by release of ^{51}Cr from labelled target cells. Target cells ($5–10 \times 10^6$) were incubated at 37° for 30 minutes in 2 ml PFB 10 containing 100 μc ^{51}Cr sodium chromate (obtained from the Radiochemical Centre at Amersham, specific activity not less than 100 μc/μg chromium). The cells were then washed four times in PFB 10 before being suspended finally at 10^5 cells/ml.

Joint-lymphocyte cytotoxicity tests involved the incubation of 10^5 target cells with $2·5 \times 10^6$ joint mononuclear cells in 2-ml PFB 10 in Falcon plastic tubes. Incubation was for 18 hours.

Cytotoxicity tests with joint fluid were performed with 3-ml volumes of PFB 10 containing 6·7 per cent joint fluid, 10^5 Chang cells and $2·5 \times 10^6$ normal human lymphocytes. Incubation was for 18 hours in Falcon plastic tubes.

With all other experiments, quantitative comparisons were made only within the experiment. Conditions within each experiment were kept rigidly constant; however, conditions varied to some extent from experiment to experiment. The ratio of lymphocytes to target cells was always of the order of 25 : 1, but the absolute numbers of these varied depending largely upon the number of human lymphocytes available. Incubation was for 18 hours in Sterilin plastic tubes.

In all these experiments incubation was at 37° in a moist atmosphere of 5 per cent CO_2 and 95 per cent air. At the end of incubation the tubes were spun at 300 ***g*** for 15 minutes at 10°. One millilitre of supernatant was then removed and by counting the ^{51}Cr activity in the 1 ml of supernatant and in the original tube the percentage of ^{51}Cr release could be calculated.

Cytotoxicity

This is defined, following the practice of Holm and Perlmann (1967a, b), as the percentage of ^{51}Cr release from target cells without lymphocytes subtracted from the percentage of ^{51}Cr release with lymphocytes.

Tritiated thymidine incorporation

Cultures containing $2{\cdot}5\times10^6$ lymphocytes in Parker 199 with serum enrichment as specified later were set up in sterile plastic tubes. These were incubated for 24 hours at 37° in a humid atmosphere of 5 per cent CO_2 and 95 per cent air before the addition of 1 μc of tritiated thymidine per millilitre. Specific activity was adjusted to 80 mc/mM with unlabelled thymidine. Incubation was stopped at 72 hours, the tubes centrifuged and the cell sediment frozen with added saline. Nucleic proteins were concentrated by the method of Vischer and Stastny (1967) with successive extractions in 0·9 M trichloracetic acid, 44 per cent dimethylsulphoxide in phosphate buffered saline and 85 per cent ethanol. The final precipitate was dissolved in 0·1 N sodium hydroxide, suspended in Bray's solution and counted in a Tracerlab liquid scintillation counter. The results are the means of triplicates and were obtained by subtracting the value obtained from cultures with tritiated thymidine added immediately before harvesting.

RESULTS

Joint fluid was taken from twenty-one consecutive patients attending the outpatient department of this hospital for knee aspiration. The effect of 6·7 per cent joint fluid on the cytotoxicity of $2{\cdot}5\times10^6$ peripheral blood lymphocytes from healthy controls towards 10^5 Chang cells was determined. Controls contained 6·7 per cent foetal bovine serum (FBS). All tubes contained culture medium with 10 per cent FBS. The cytotoxicity of $2{\cdot}5\times10^6$ joint fluid mononuclear cells on 10^5 Chang cells was also determined. The results of these

TABLE 1

LACK OF CORRELATION IN DONORS, BETWEEN CYTOTOXICITY-INDUCING EFFECTS OF CERTAIN JOINT FLUIDS AND THE PRESENCE OF JOINT MONONUCLEAR CELLS WITH HIGH CYTOTOXICITY

Joint fluid donor	Diagnosis and Latex test	Deviation of cytotoxicity in joint fluid from that in FBS	Cytotoxicity on joint mononuclears
R.D.	R.A. −ve	+0·4	8·0
M.S.	R.A. +ve	−8·8	ND
R.K.	R.A. −ve	+5·8	2·8
E.R.	R.A. +ve	−0·5	22·1
S.W.	R.A. −ve	−6·9	8·1
A.M.	R.A. +ve	−4·7	25·2
P.M.	Still's −ve	−6·8	25·0
V.C.	R.A. +ve	+22·5	13·9
L.N.	R.A. +ve	−3·9	12·2
J.K.	R.A. −ve	−6·7	8·0
E.C.	Still's −ve	+0·9	2·5
F.J.	R.A. +ve	+2·5	ND
C.W.	Still's −ve	−1·2	ND
G.R.	Still's −ve	−10·7	3·6
P.B.	R.A. −ve	+3·9	6·3
F.E.	R.A. +ve	+0·9	3·4
J. F.	R.A. −ve	+0·2	ND
P.M.	Still's −ve	−3·6	8·0
A.L.	R.A. −ve	−2·0	12·5
− T.	R.A. −ve	+5·8	19·8
K.F.	R.A. −ve	+16·6	9·4

The effect of 6·7 per cent joint fluid was compared with that of 6·7 per cent foetal bovine serum (FBS). In all tubes PFB 10 was used as basic culture medium. There were $2{\cdot}5\times10^6$ mononuclear cells and 10^5 Chang cells in each tube. None of the joint fluids affected the spontaneous release of ^{51}Cr from Chang cells.

experiments are shown in Table 1. Three patients showed joint mononuclear cell cytotoxicity above the normal range for peripheral blood lymphocyte cytotoxicity as determined by MacLennan and Loewi (1968a).

Four patients possessed fluid which increased the cytotoxicity of blood lymphocytes by more than 5 cytotoxicity units above that shown with foetal bovine serum.

Seven patients possessed fluid which depressed cytotoxicity by more than 25 per cent of the cytotoxicity shown by control tubes.

Two of the patients with high joint mononuclear cell cytotoxicity had fluid which decreased the spontaneous cytotoxicity of normal lymphocytes. There was clearly no positive correlation, however, between patients showing increased joint mononuclear cell cytotoxicity and patients with fluid which increased the cytotoxicity of normal blood lymphocytes.

REPRODUCIBILITY OF THE PROPERTY OF CERTAIN JOINT FLUIDS TO INCREASE CYTOTOXICITY

Table 2 shows the effect of joint fluids from one patient (K.F.) taken at different times on the cytotoxicity of normal lymphocytes from the same donor. The ability of a single fluid to increase the cytotoxicity of lymphocytes from a number of donors is demonstrated. It is shown that serum taken from this patient also has the ability to increase cytotoxicity.

TABLE 2

INCREASE IN CYTOTOXICITY INDUCED BY SERUM AND JOINT FLUID FROM A SINGLE DONOR

Lymphocyte donor	Cytotoxicity		Origin of fluid	Date of fluid extraction (1968)
	FBS 3 per cent	Donor serum or joint fluid 3 per cent		
D.R.P.	7·1	47·2	Right knee	25th April
J.W.	18·0	40·8	Right knee	25th April
B.T.	19·6	35·2	Right knee	25th April
B.T.	19·6	35·9	Left knee	25th April
G.L.	18·5	57·0	Serum	21st May
G.L.	18·5	56·7	Left knee	21st May
I.C.M.M.	19·0	38·8	Serum	2nd December

INCIDENCE OF CYTOTOXICITY-INCREASING FACTOR IN SERUM FROM SEVENTY-EIGHT DONORS

Three per cent serum, from these donors, in 2 ml PFB 10 was added to tubes containing $2{\cdot}5 \times 10^6$ blood lymphocytes from healthy human donors and 10^5 Chang cells. Of the serum donors, fifty-two had chronic inflammatory joint disease, five had neoplasia, two had infections, one had abdominal pain of uncertain origin, and eighteen were healthy controls.

Twelve subjects out of the seventy-eight tested have been found to have serum which appreciably increases the cytotoxicity of normal lymphocytes towards Chang cells. In all these cases the effect is consistently observed although the highest dilution of serum or fluid producing increased lymphocyte cytotoxicity sometimes alters with time. Serum with the ability to increase cytotoxicity has mainly been found in patients with chronic inflammatory joint disease although one patient, with acute abdominal pain of uncertain origin which resolved spontaneously within a few days, was found to have serum with this effect, as was one healthy control.

The incidence of patients with serum able to increase cytotoxicity may not represent the incidence of cytotoxicity-increasing factor, for as will be shown later, cytotoxicity increasing factors and factors which reduce ^{51}Cr release from target cells may coexist in the same serum. Factors which result in decreased ^{51}Cr release appear to be in the main not specific for the target cell and are only noticeable in serum concentrations in excess of 3 per cent providing the basic culture medium contains 10 per cent FBS. Certain globulin fractions can result in marked reduction of cytotoxicity. It is possible that these may in part act specifically on Chang cells, protecting them from damage by lymphocytes but not in themselves inhibiting ^{51}Cr release. Further analysis of these factors which protect Chang cells must be made before firm conclusions can be drawn. This paper deals in detail only with the factor which renders Chang cells susceptible to increased lymphocyte damage. The investigations which follow have been performed on two pools of sera from two patients possessing this factor. These two pools will be called FCS 1 and FCS 2. Foetal bovine serum (Flow batch 40023), FBS and pooled normal human serum (PNHS) were the control sera.

CHEMICAL IDENTIFICATION OF THE FACTOR INCREASING LYMPHOCYTE MEDIATED CELL DAMAGE TO CHANG CELLS

On the basis of molecular size

The active factor was not dialysable and was entirely precipitated from solution by half saturation with ammonium sulphate. Separation on Sephadex G-150 showed (Fig. 1), on the basis of equal optical density solutions, at least 100 times more activity in Peak II (11–7S) than in Peak I (19S) and Peak III (albumin).

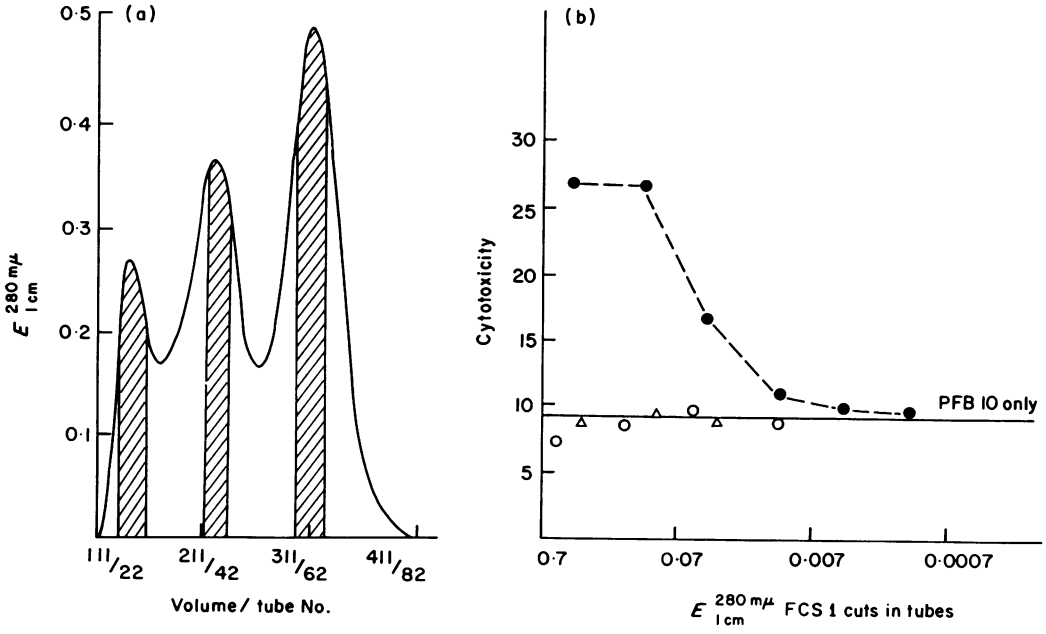

FIG. 1 (a) Separation of FCS 1 (1 ml) on Sephadex G-150. Hatched columns, fractions analysed. (b) The effect of fractions of FCS 1 from Sephadex G-150 on lymphocyte-mediated Chang cell damage. △, Peak I; ●, Peak II; ○, Peak III.

With DEAE-cellulose

FCS 1 was adsorbed on DE 52 equilibrated with 0·01 M phosphate buffer at pH 7·3. Successive elution with 0·01, 0·025, 0·05 and 0·15 M buffers produced eluates with activities as shown on Fig. 2. The 0·01 M cut was seven to ten times more active on the basis of equal optical density solutions than whole serum or the 0·025 M cut. The 0·05 and 0·15 M cuts contained at least 100 times less activity than whole serum, also on the basis of solutions of equal optical density.

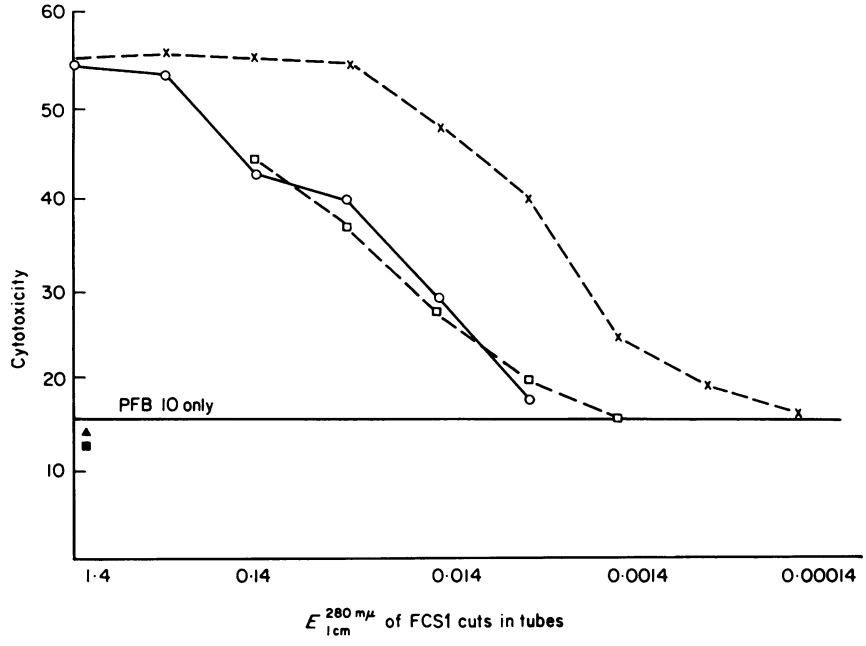

FIG. 2. Enrichment of cytotoxicity-increasing factor of FCS 1 by separation on DE 52 cellulose at pH 7·5. ×, 0·01 M cut; □, 0·025 M cut; ▲, 0·05 M cut; ■, 0·15 M cut; ○, whole serum.

Separation of the 0·01 M *fraction from DE* 52 *on an ampholine pH gradient*

FCS 1 and FCS2 0·01 M DE 52 cuts and Cohn Fraction II of PNHS were separated in this way. The components of FCS 1 and FCS 2 which are active in increasing cytotoxicity were slow-moving. The protein moving with ampholines between pH 5·5 and 7·5 inhibited lymphocyte cytotoxicity although none of the fractions had any effect, in the concentrations used, on spontaneous ^{51}Cr release from Chang cells. Fig. 4 shows the protein concentration of the various cuts and this clearly indicates that the component that increases lymphocyte mediated cell damage, only represents a small part of the total IgG. FCS 1 and FCS 2 were both stable at pH 5 and pH 10. Dialysis of FCS 2 and FCS 1 against buffers at these pH levels for 2 days at 4° did not result in any loss in activity compared with FCS 1 and FCS 2 dialysed against buffer at pH 7·2. The above analysis indicates that the component in FCS 1 and FCS 2 that increases lymphocyte cytotoxicity to Chang cells is an IgG or at least separates with IgG. Concentrated active cuts from isoelectric focusing formed a precipitin line with anti-human IgG.

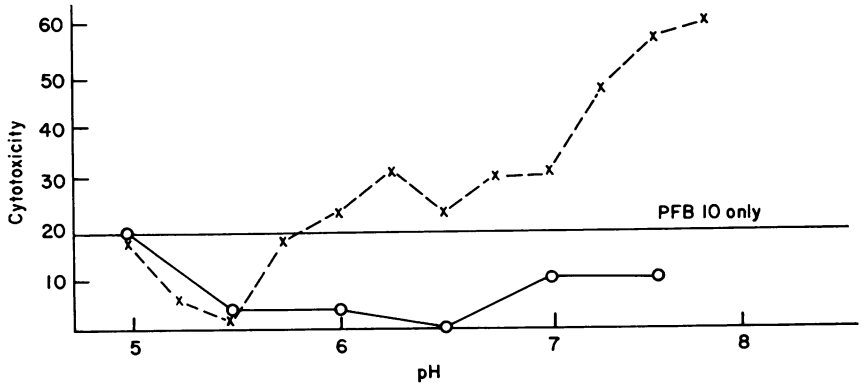

FIG. 3. Effect on cytotoxicity of fractions from isoelectric focusing. ×, 0·01 M cut from DE 52 of FCS 1; ○, Cohn Fraction II of pooled normal human serum. Cuts from the isoelectric focusing column were added to give a concentration of $E^{280m\mu}_{1cm}=0{\cdot}09$. The tubes also contained 2×10^6 lymphocytes and 10^5 Chang cells in 2 ml PFB 10 and 1 ml 1·5 M phosphate buffer, pH 7·2.

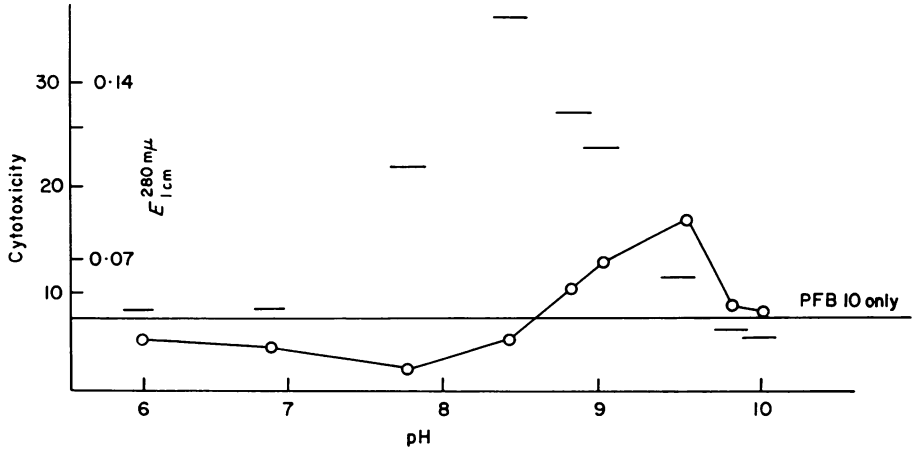

FIG. 4. Effect on cytotoxicity of fractions from isoelectric focusing: ○, 0·01 M cut from DE 52 of FCS 2. Fractions from the isoelectric focusing column were added to give a concentration of $E^{280m\mu}_{1cm}$ 0·045. Horizontal lines (—) represent the total protein concentration in each fraction from isoelectric focusing column. The tubes also contained $1{\cdot}7\times10^6$ lymphocytes and 10^7 Chang cells in 2 ml PFB 10 and 1 ml 1·5 M phosphate buffered saline.

THE EFFECT OF COMPLEMENT ON CHANG CELL SURVIVAL IN THE PRESENCE OF FCS 1

Levels of FCS 1 as shown in Table 3 that are capable of inducing increased Chang cell damage in the presence of normal human lymphocytes did not, *per se*, damage Chang cells even in the presence of 10 per cent fresh guinea-pig serum. Rabbit anti-Chang antibody completely lysed Chang cells in the presence of 10 per cent fresh guinea-pig serum, but was non-toxic in the presence of heat inactivated guinea-pig serum. Rabbit anti-Chang antibody was able to increase human lymphocyte damage to Chang cells in the absence of complement. This effect was seen at dilutions of antiserum of $1:10^6$ while complement-dependent lysis required antiserum concentrations in excess of $1:10^3$. These findings are incompatible with the suggestion of Taylor and Culling (1968) that increased target cell damage by lymphocytes in the presence of target cell specific antibody results from *in vitro* synthesis of complement.

TABLE 3

THE EFFECT OF (a) COMPLEMENT, AND (b) NORMAL HUMAN LYMPHOCYTES ON THE PER CENT ^{51}Cr RELEASE FROM CHANG CELLS IN THE PRESENCE OF EITHER 3 PER CENT FCS 1 OR 3 PER CENT RABBIT ANTI-CHANG ANTISERUM

	Per cent ^{51}Cr release		
	10 per cent inactivated guinea-pig serum	10 per cent fresh guinea-pig serum	Normal human lymphocytes
Chang cells	22·4	22·9	35·4
Chang cells + 3 per cent FCS 1	22·1	21·8	57·0
Chang cells + 3 per cent rabbit anti-Chang antiserum	22·6	76·4	58·7

SITE OF ACTION OF FCS 1

Chang cells 10^6 and normal lymphocytes $2{\cdot}5 \times 10^7$ were independently pre-incubated in 10 ml of PFB 10 containing 1 : 500 FCS 1 for 30 minutes at 37°. The cells were then washed three times in PFB 10 before being set up with Chang cells or lymphocytes which had not been pre-incubated with FCS 1. Lymphocytes pre-incubated with FCS 1 were no more toxic to untreated Chang cells than untreated lymphocytes. Pre-incubated Chang cells, however, were more susceptible to lymphocyte damage than Chang cells which had not been pre-incubated with FCS 1 (see Table 4).

TABLE 4

THE EFFECT ON CYTOTOXICITY OF PRE-INCUBATING CHANG CELLS AND LYMPHOCYTES WITH FCS 1

	Cytotoxicity	
	Experiment 1	Experiment II
Chang cells + lymphocytes	8·6	19·1
Lymphocytes pre-incubated in FCS 1 + Chang cells	9·6	19·6
Chang cells pre-incubated in FCS 1 + lymphocytes	17·0	52·2
Chang cells + lymphocytes + FCS 1	34·3	57·2

Pre-incubation was for 30 minutes at 37° with FCS 1 with 10^5 Chang cells/ml or $2{\cdot}5 \times 10^6$ lymphocytes/ml.

ABSORPTION OF FCS 1 CYTOTOXICITY INCREASING FACTOR ONTO CHANG CELLS

Chang cells (0·25 ml) and horse red blood cells (0·25 ml) were each incubated in the presence of 5 ml of PFB 10 containing 1 mg of Cohn Fraction II from PNHS for 30 minutes in order to saturate the cells with normal human γ-globulin and reduce non-specific absorption of the active factor in FCS 1. The cells were then washed twice in PFB 10 before incubation at 37° in 10 ml of PFB 10 containing 1 : 300 FCS 1 for 1 hour with occasional mixing. At the end of this time the cell suspensions were spun down and the supernatants were filtered. The supernatant incubated with horse red cells had lost less than 10 per cent of its ability to increase lymphocyte mediated cell damage while the supernatant which had been incubated with Chang cells had lost 90 per cent of its original activity.

SPECIFICITY OF ACTION OF CYTOTOXICITY INCREASING FACTOR

Human lymphocytes were set up against ^{51}Cr-labelled target cells from different sources in the presence or absence of FCS 1. Diploid human fibroblasts could only be obtained as single cell suspensions after treatment with 0·25 per cent trypsin in Dulbecco calcium-free buffer for 20 minutes. All other cells were grown by the method used for Chang cells.

The cell lines used were: (1) Chang cells, a polyploid strain derived from normal human liver; (2) McCoy cells, a polyploid strain derived from a human osteoarthritic knee effusion; (3) L strain fibroblasts, a polyploid strain of C3H mouse fibroblasts; and (4) Flow 2000, a diploid strain of human foetal fibroblasts.

Of these cell lines only Chang cells were susceptible to appreciably increased damage by lymphocytes in the presence of FCS 1 (see Table 5).

TABLE 5

THE EFFECT OF FCS 1 ON NORMAL HUMAN LYMPHOCYTE CYTOTOXICITY TO DIFFERENT TARGET CELLS

	Per cent ^{51}Cr release			
	Chang cells	L cells	McCoy	Flow 2000
PFB 10	21·9	29·8	25·5	22·2
PFB 10 1 : 100 FCS 1	23·0	30·1	26·6	ND
Human lymphocytes + PFB 10	31·3	39·3	30·6	32·3
Human lymphocytes + PFB 10 + 1 : 100 FCS 1	57·3	38·3	33·5	32·5

THE EFFECT OF FCS 1 AND FC JOINT FLUID 1 ON TRITIATED THYMIDINE INCORPORATION BY AUTOLOGOUS LYMPHOCYTES AND LYMPHOCYTES FROM A HEALTHY CONTROL

Lymphocytes from the donor FCS 1 and a normal healthy control were cultured in the presence of FCS 1 and FC joint fluid 1 (Table 6). Tritiated thymidine incorporation by lymphocytes from both donors was not appreciably affected by FCS 1 or FC joint fluid 1 although both responded to phytohaemagglutinin. The same batches of lymphocytes, however, showed markedly increased cytotoxicity to Chang cells in the presence of FCS 1 and FC joint

TABLE 6

FAILURE OF SERA AND JOINT FLUIDS WHICH ARE ABLE TO INCREASE THE CYTOTOXICITY OF LYMPHOCYTES TO CHANG CELLS TO INFLUENCE THE INCORPORATION OF TRITIATED THYMIDINE INTO AUTOLOGOUS OR HOMOLOGOUS LYMPHOCYTES

Serum additive	Lymphocytes from healthy control		Autologous lymphocytes	
	72 hours ^{3}HTdR uptake (counts/min)	18 hours cytotoxicity to Chang cells	72 hours ^{3}HTdR uptake (counts/min)	18 hours cytotoxicity to Chang cells
FBS 10 per cent	136	18·5	2,697	9·9
FBS 3·3 per cent 6·7 FCS 1 per cent	336	57·0	2,217	33·2
FBS 3·3 per cent 6·7 per cent FC joint fluid	280	56·7	2,132	26·7
FBS 10 per cent + PHA M 0·005 ml/ml	16,619	41·5	29,770	ND

^{3}HTdR, tritiated thymidine; PHA M, phytohaemagglutinin M (Difco).

fluid 1. These results would suggest that FCS 1 does not act by directly stimulating lymphocytes. They do not exclude the possibility that FCS 1 anti-Chang antibody and Chang cells together might produce a mitotic response in non-immune lymphocytes. Such an effect by antigen–antibody complexes has been shown (Moller, 1969).

DISCUSSION

The data which have been presented in this paper demonstrate the presence of a factor in twelve out of seventy-eight human sera tested which renders a polyploid strain of human liver cells susceptible to greatly increased damage by non-immune human lymphocytes. This factor has considerable specificity in that it fails to induce increased non-immune lymphocyte damage to other homologous and heterologous target cells tested. It is also specifically absorbed on Chang cells. Chemically the substance behaves as IgG. With regard to both its specificity of action and chemical properties, therefore, it is an IgG antibody which becomes attached to an antigen on the Chang cell surface. There are a number of possibilities to account for the nature of this antigen. It could be a normal tissue antigen; an antigen acquired from the culture medium; or an antigen which had arisen at the time of genetic alteration during Chang cell propagation in culture. The first possibility might arise if the Chang cell carried a normal human transplantation antigen, against which antibodies might be formed by patients who had, for instance, received a blood transfusion. In our material only two out of twelve subjects with this serum antibody had had blood transfusions. Another possibility is that an antibody directed against liver and thus Chang cells might have arisen in patients suffering from liver disease. Only one of the patients gave a history suggestive of liver disease. The second suggestion is discouraged by the fact that other cell lines propagated in identical medium did not bind the antibody. It is easier to explain the data relating to this antigen–antibody system on the basis of the third possibility. Cells are known to acquire virus-specific antigens after infection by certain viruses. Such antigens may be manifested on the cell surface and are transmitted as a heritable factor (Hellström and Moller, 1965; Sjogren, 1965; Klein, 1965; Rowe, 1967). Different cell lines infected with the same virus carry common antigens. Transformation induced by non-viral agents, such as methylcholanthrene or radiation does not result in the appearance of predictable cell antigens. Unless the antibody described in this paper fortuitously cross-reacts with a Chang cell antigen, the above arguments would favour the conclusion that a Chang cell antigen was virus-induced and that patients possessing antibody have been infected by that virus, or a related virus.

The presence of factors in certain sera and joint fluids which decrease target cell damage might have indicated the presence of specific antibody which enhances target cell viability. The inhibition of target cell damage, however, appears complex. So far specific inhibition of target cell damage has not been clearly demonstrated in this system and further analysis is required.

The experimental data presented above fail to demonstrate any evidence of target cell specific antibody in the joint fluids of patients who possess abnormally cytotoxic joint mononuclear cells. The finding of serum factor specific for Chang cells in a patient without joint disease and in a healthy control discourages any conclusion that such a factor may be of pathogenic significance in joint disease. As factors which enhance target cell survival and antibody inducing lymphocyte mediated cytotoxicity to target cells can coexist in the same serum, simple screening of sera for the latter is impossible. Much

more detailed evidence will have to be collected before correlating the presence of such factors with any pathological process.

Lundgren, Collste and Möller (1968) have pointed to a possible role of antibody which renders target cells susceptible to lymphocyte mediated cell damage in delayed hypersensitivity and graft rejection. Such antibody might also be found in response to tumour-specific antigens. Apart from the possible significance of such antibodies in tumour rejection, they are likely to be of considerable use in detecting allergic response to tumours, for this system is at least 1000 times more sensitive than complement fixation, and such antibody is active at levels well below the sensitivity of fluorescent antibody techniques. In our hands, attempts to detect FCS 1 on Chang cells with fluorescent anti-human IgG have failed, suggesting that the number of combining sites on the Chang cell is few, which indicates that the amount of antibody required to induce lymphocyte damage is small. It must, nevertheless, be noted that there is so far no evidence showing that the mechanism described in this paper has a counterpart *in vivo*. In any actual allergic response it is likely to play only a part in a complicated system of several immunological mechanisms some of which may be influencing the final result in opposing ways.

ACKNOWLEDGMENTS

The authors would like to thank Dr J. E. Scott and Dr L. E. Glynn for helpful advice and criticism. The skilled technical assistance of Mr B. Harding is also gratefully acknowledged.

I.C.M.MacL. is in receipt of a grant from the Arthritis and Rheumatism Council.

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