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THE RESPONSE OF LYMPHOCYTES FROM NON-IMMUNIZED HUMANS TO ANTIGEN–ANTIBODY COMPLEXES*

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SUMMARY

The stimulatory effect of antigen–antibody–complement complexes on cultured normal human peripheral blood lymphocytes was studied. The donors of the cells had not been sensitized to the antigens used. Two antigens were used: flagellar antigen of *Salmonella paratyphi* B (SPB) and bovine serum albumin (BSA), with their respective antibodies prepared in rabbits. The addition of such antigen–antibody aggregates to the cultures stimulated the lymphocytes as determined by morphological changes and increased uptake of [^{14}C]thymidine into DNA. Peak of stimulation was observed after 5–6 days of culture incubation. The stimulation appeared to be complement dependent. The lymphocytes showed no response either to the antigen alone or to anti-SPB. When BSA–anti-BSA was centrifuged, most of the stimulatory activity was found in the supernate. The most likely explanation of this stimulation is injury to lymphocyte membranes, possibly from a non-specific attachment of immune complexes to them. A similar mechanism of membrane injury may underlie reactions to all non-specific stimulants, and possibly also to specific antigens to which the cell donor is sensitized.

INTRODUCTION

Human peripheral blood lymphocytes show little metabolic activity when cultured *in vitro*. However, they may be stimulated to enlarge and divide in culture. During the early part of this response, new RNA (Cooper & Rubin, 1965), probably including messenger, transfer and ribosomal types, is synthesized. At the same time new proteins, about 5% of which is γ-globulin (Ripps & Hirschhorn, 1967), are produced, whereas later in culture

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new DNA is synthesized. Such stimulation can be caused either by non-specific agents (Nowell, 1960) or by specific antigens (Hirschhorn *et al.*, 1963a).

The stimulation of lymphocytes from sensitized donors by the specific sensitizing antigen is probably due to its attachment to an antibody site on the cell membrane. The mechanism by which non-specific agents, such as phytohaemagglutinin and several other plant products stimulate lymphocytes, even those obtained from agammaglobulinaemics (Fudenberg & Hirschhorn, 1964) and newborn infants, is not understood. However, it is known that such stimulation can also be produced by substances that are membrane damaging, such as streptolysin S (Hirschhorn *et al.*, 1964) and staphylococcal toxins (Ling & Husband, 1964). These findings led to the hypothesis that the initiating event in lymphocyte stimulation is the attachment of various types of macromolecules to the cell membrane, possibly with minor injury.

Antigen–antibody–complement complexes are known to bind to the surface of small lymphocytes (Uhr, 1965) and to be capable of damaging red cell membranes. The capacity of such aggregates to stimulate lymphocytes was studied.

MATERIALS AND METHODS

Preparation of cultures

Pints of heparinized venous blood were obtained from normal donors. The blood was sedimented in plastic tubes in a 37°C water bath, the tubes being placed at a 45° angle. After about 3 hr, the lymphocyte-rich upper portion of the supernatant plasma was pipetted off into disposable plastic centrifuge tubes. Cells were centrifuged at 165 *g* in an International Centrifuge, Universal Model UV, for 10 min, resuspended in Eagle's minimum essential medium (MEM, Grand Island Biological Co., Grand Island, New York) (Eagle, 1959) modified for suspension cultures and washed twice. Cell counts were performed, including viability counts, using 0·134% Erythrocin B mixed in 1:1 ratio with the cell suspensions. Five ml cultures containing 4×10^6 cells/culture were set up. The culture medium was composed of MEM with 30% normal or immune rabbit serum, 1% of 200 mM fresh glutamine (Grand Island Biological Co.), 100 μg penicillin (Grand Island Biological Co.) and 100 μg streptomycin/ml (Grand Island Biological Co.) with or without the additives to be studied. Except when eliminated for the purpose of the experiment, at least one-third of the serum was normal fresh rabbit serum in order to provide complement. Cultures were incubated in a 37°C water bath, usually for 5–6 days. Twenty-four hours before harvesting, 0·15 μc of [^{14}C]thymidine (New England Nuclear Corp., Boston, Massachusetts) (S.A.:30 mc/mM) was added per each millilitre of culture.

Harvesting

Cell suspensions were transferred to glass centrifuge tubes and centrifuged at 370 *g* for 15 min. Cells were resuspended and washed three times with normal saline. After washing, cells were alternately frozen in dry ice–acetone and thawed seven times. The cell homogenate was suspended in 2 ml of cold 15% TCA. Proteins and DNA were precipitated overnight at 4°C. The TCA precipitates were centrifuged at 1600 *g* for 10 min, and the pellet was washed once with cold 15% TCA. The pellet was dissolved in 0·5 ml of hyamine (Packard, hydroxide of hyamine, 10-X, 1M, Downers Grove, Illinois), left for a short time at 37°C, added to 10 ml of Bray's solution (Bray, 1960) and counted in a scintillation counter

(Packard Tricarb Spectrophotometer, Model 3375). All measurements were done in duplicate and background samples consisted of Bray's solution + hyamine, without cells. Counts were corrected for quenching and counting efficiency of the instrument by the channels-ratio method (Bush, 1963). Traces of water which were present in the samples increased the efficiency of scintillation counting in agreement with previous observations (Moorhead & McFarland, 1966).

In two of the experiments with the SPB–anti-SPB system, the morphology of the lymphocytes was studied. Cultures for morphological studies were set up exactly as described before. Four hours before harvesting, 0·1 ml of 0·5 μg/ml vinblastine sulphate (Velban, Eli Lilly, Indianapolis, Indiana) was added to each culture. Cells were spun down and resuspended in hypotonic solution (1% sodium citrate), fixed in 3:1 methanol–acetic acid for 10 min at room temperature and then placed on a cover slip, air dried and stained with 0·5% acetic orcein. Slides were examined with phase illumination, and quantitation of response was scored by classifying at least 1000 cells as large, small or in mitosis. Degree of response is defined as the percentage of small lymphocytes that were morphologically transformed to large cells, some of which resembled plasma cells, plus mitoses, in excess of the percentage found in the control cultures with no additive. Normally, cultures with no additive show from 5 to 15% stimulated cells by 3 days and up to 20% by 6 days. A difference of 5% in 1000 cells is statistically significant.

BSA–anti-BSA complex was also prepared in the absence of cells in order to test the stimulatory activity of the precipitable complex and any product released from the interaction of this complex and the complement into the supernate. Normal fresh rabbit serum and the antigen–antibody complex were incubated in a 37°C water bath for 15 min, left at 4°C for 1 hr and centrifuged at 580 *g* for 10 min. The precipitate was resuspended and washed twice with normal saline.

RESULTS

SPB–anti-SPB complex

Table 1 shows the results of microscope examination for cell morphology. There was no difference between the levels of lymphocyte response in control cultures and cultures with SPB antigen. PHA, as expected (Hirschhorn *et al.*, 1963b), produced a high percentage of enlarged lymphocytes and mitoses. The addition of SPB–anti-SPB complex resulted in a higher level of enlarged lymphocytes as compared with the control cultures. This stimulation was detected maximally after 6 days of incubation.

The same type of result was obtained with [^{14}C]thymidine uptake, used as a criterion of DNA synthesis. There was essentially no change in the uptake of [^{14}C]thymidine in cultures with antigen alone or antibody alone as compared with cultures having no additive. Phytohaemagglutinin produced its known stimulation of DNA synthesis, and addition of antigen–antibody–complement complex resulted in a significant increase in thymidine uptake.

Out of eleven experiments, each of a different normal blood donor, ten experiments showed an increase of thymidine uptake in the presence of the complex. The average ratio of DNA-associated radioactivity in cultures containing the complex compared to cultures with antigen alone was 9·43 with a range of 2·1–32·2. P value by paired t-test was $<0{\cdot}025$ (Table 2).

Different doses of antigen and antibody were used, ranging from 0·3 to 30·0 μg of antigen,

TABLE 1. Lymphocyte response to SPB–anti-SPB complex

Additive	Incubation period (days)	Response*
Donor A		
None	3	14
PHA	3	82
SPB	3	10
Complex	3	11
None	6	17
PHA	6	83
SPB	6	21
Complex	6	31
Donor B		
None	6	5
SPB	6	7
Complex	6	13

* % of large cells + % of mitoses/1000 cells.

and from 0·1 to 1·0 ml antiserum/culture. As shown in Fig. 1, the optimal stimulatory dose of complex contained 30·0 μg of flagellar antigen and 1·0 ml of rabbit anti-flagellar antiserum, which had an immobilization titre of 1:100,000.

Cultures containing the complex were incubated for several periods, from 1 to 6 days. The time of maximal stimulation above the levels observed for the antigen alone was on the 5th day of incubation. Accordingly, the cultures in these experiments were harvested on the 5th

TABLE 2. SPB–anti-SPB complex

Experiment No.	No additive	PHA	Ab	Ag	Complex	Complex/Ag ratio	Ab/Ag ratio
1	188	—	—	166	355	2·1	—
2	109	—	—	155	340	2·2	—
3	—	90,106	—	134	950	7·1	—
4	—	—	—	162	1206	7·4	—
5	—	42,000	—	162	3036	18·7	—
6	—	—	—	132	1926	14·6	—
7	362	76,900	416	166	5352	32·2	2·5
8	240	91,780	160	114	540	4·7	1·4
9	—	—	180	176	514	2·9	1·0
10	170	10,299	—	165	396	2·4	—
Mean						9·43	1·63
SE						±3·09	±0·45

All counts are reported as disintegrations per minute (dpm).

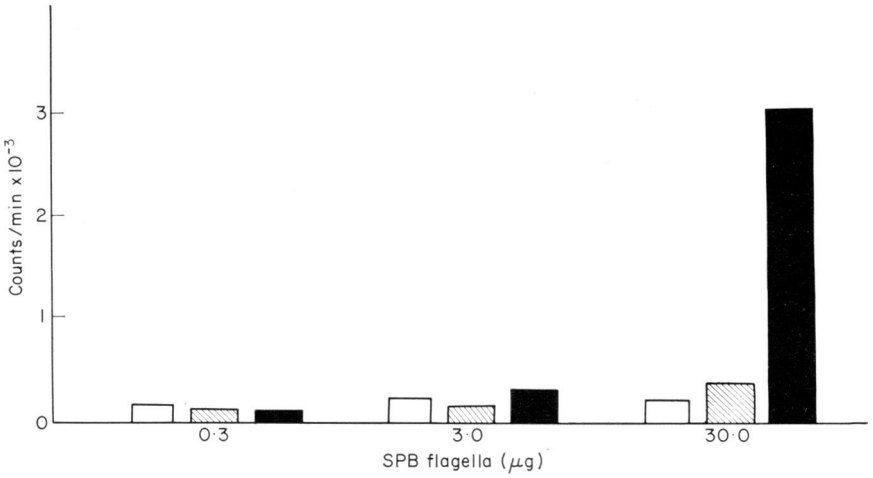

FIG. 1. SPB–anti-SPB dose curve (83% counting efficiency). Open columns, No Ab; hatched columns, 0·1 ml Ab; solid columns, 1·0 ml Ab.

day. These results correlate well with the time of maximal stimulation derived from microscopic examination.

In order to determine whether the response of small lymphocytes to antigen–antibody complexes is associated with gross damage to the cells, viability counts were performed at zero time, 1, 3, 24, 48 and 72 hr after incubation. As shown in Fig. 2, the percentage of viable cells was not significantly different in the cultures with no additive, antigen alone or complex. In two experiments, viability counts were made up to 140 hr after incubation. There was no decrease observed and there were not significant differences in the cultures with no additive,

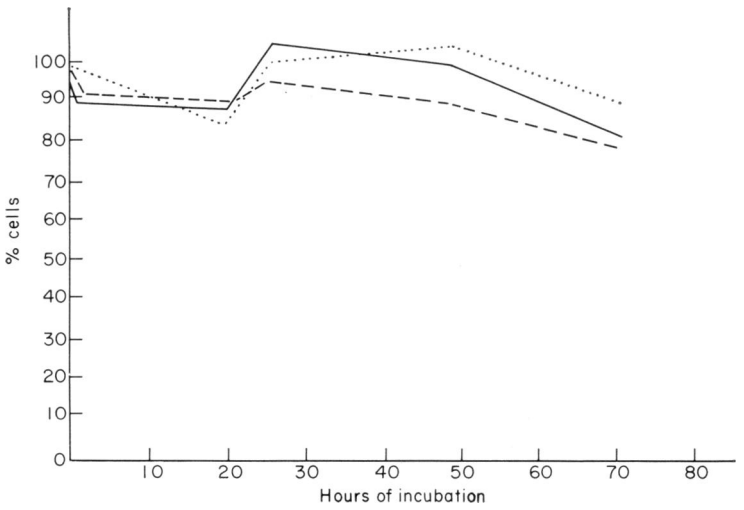

FIG. 2. Viable cells related to original cell count. – – –, SPB–anti-SPB; -----, SPB; ——, no additive.

antigen alone or complex. It may thus be concluded that the activation of cells by the complex is not followed by a significant increase in cell death.

The necessity for the presence of complement in the stimulation by antigen-antibody complexes was investigated by comparing [^{14}C]thymidine uptake in cultures containing normal fresh rabbit serum with cultures containing 'aged' rabbit serum (serum stored at 4°C for at least 1 month).

In the experiment shown in Table 4, there was a 4·5-fold increase of incorporation of

TABLE 3. BSA–anti-BSA complex

Experiment No.	No additive	PHA	Ab	Ag	Complex	Complex/Ag ratio	Ab/Complex ratio	Ab/Ag ratio
1	—	139,120	—	160	10,960	68·5	—	—
2	—	81,810	—	180	14,640	81·3	—	—
3	—	193,000	—	160	23,320	145·7	—	—
4	145	20,510	—	163	1,358	8·3	—	—
5	100	—	—	103	854	8·3	—	—
6	100	10,080	—	260	5,980	23·0	—	—
7	134	15,800	1,332	208	1,146	5·5	1·2	6·4
8	—	—	—	104	795	7·6	—	—
9	—	—	—	140	1,408	10·1	—	—
10	105	20,545	7,940	145	9,500	67·8	0·8	54·8
11	—	—	—	156	5,702	36·6	—	—
12	—	—	—	126	2,727	21·6	—	—
13	170	87,080	12,190	180	15,970	88·7	0·8	67·7
14	—	—	—	160	9,670	60·4	—	—
15	—	—	—	170	9,980	58·7	—	—
16	217	75,290	693	103	792	7·7	0·9	6·7
17	158	31,330	1,843	214	1,427	6·7	1·3	8·6
18	305	26,700	—	231	1,818	7·9	—	—
19	293	17,820	1,033	139	645	4·6	1·6	7·4
Mean						37·84	1·10	25·27
SE						±9·00	±0·13	±11·50

All counts are reported as dpm.

radioactive material in the presence of complex as compared to antigen alone when cultures were prepared with normal fresh rabbit serum. No increase was observed in the presence of aged rabbit serum.

BSA–anti-BSA complex

Crystalline bovine serum albumin and its antiserum made in rabbits was used. In nineteen experiments performed with BSA–anti-BSA complex, all showed stimulation. The average increase in thymidine uptake was 37·8 times the control levels, with a range of 4·6–145·7 (BSA alone was considered as the control). Paired *t*-test reveals a *P* value of $<0·005$ (Table 3). While BSA alone produced no stimulation, a rather high stimulation was observed with the addition of anti-BSA alone to cultures. The average increase in six experiments with anti-BSA was 25·3 (range: 6·4–67·7) times the control levels, which is not significantly lower than the increase observed with the complex.

In order to test whether this increase of thymidine uptake was due to a direct interaction of anti-BSA with the lymphocyte membrane, absorption experiments were performed. The antiserum was absorbed with fresh lymphocytes from the same donor used for the experiment with the complex. The proportions used were about $7–10 \times 10^6$ lymphocytes/1 ml of

TABLE 4. The effect of complement on the stimulation by complexes of BSA with either absorbed or unabsorbed anti-BSA

	Serum	No additive	PHA	Absorbed Ab	Ab	Ag	Complex*
SPB–anti-SPB:							
	NFRS	—	6,597	—	—	109	492
	Aged	—	9,387	—	—	196	112
BSA–anti-BSA:							
	NFRS	280	11,600	200	896	220	2,300
	Aged	200	9,600	120	110	180	88

All counts are reported as dpm.

* Made with absorbed antiserum.

antiserum with incubation for at least 4 hr at 37°C, followed by centrifugation for 15 min at 580 *g*. Three experiments summarized in Fig. 3 show that when the antiserum was absorbed in this way, the stimulation with anti-BSA was abolished and was not significantly higher than the control. On the other hand, antigen–antibody complex using absorbed antiserum retained full stimulatory capacity.

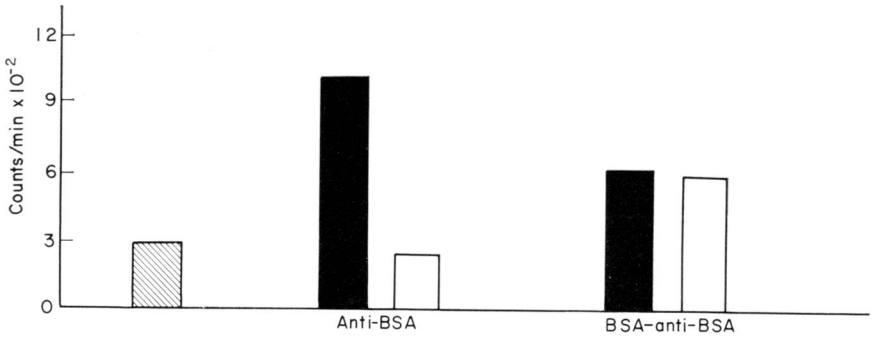

FIG. 3. Anti-BSA absorption with lymphocytes (85% counting efficiency). Open columns, Ab absorbed with lymphocytes; hatched column, no additive; solid columns, Ab not treated.

Different doses of antigen and antibody were used for stimulation. As shown in Fig. 4, the optimal stimulation occurred after the addition of 50 μg antigen and 240 μg (0·3 ml) antibody. This ratio of antigen and antibody is close to equivalence, with a slight antigen excess, as determined by quantitative precipitation.

In order to find the time of maximum stimulation, cultures with complex were incubated

for 1–6 days. Peak of stimulation was shown on the 6th day, which is approximately the same incubation period as was observed for the SPB–anti-SPB complex.

The stimulation by BSA–anti-BSA complex appears to be complement dependent like the stimulation by SPB–anti-SPB complex. In six experiments, cells were cultured both in 'aged' and in normal fresh rabbit serum. The stimulatory activity of the complex was observed only in the presence of normal fresh rabbit serum. In a typical experiment shown in Table 4, thymidine uptake in cultures containing normal fresh rabbit serum showed a 10·4-fold increase of incorporation of radioactive material in the presence of complex, as compared to antigen alone. There was no increase in the presence of complex when the lymphocytes were cultured with aged rabbit serum.

Since these experiments were done both with antiserum absorbed with lymphocytes and untreated antiserum (these data are not included in Fig. 3), it was interesting to observe that the high stimulation caused by unabsorbed anti-BSA alone was also complement dependent.

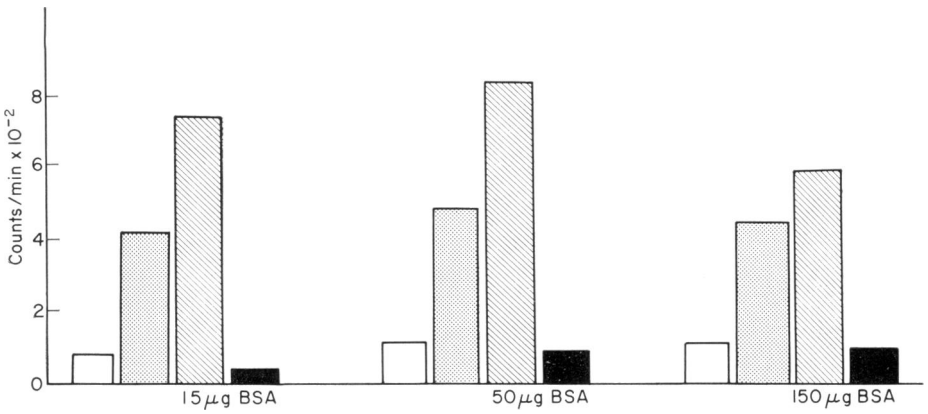

FIG. 4. BSA–anti-BSA dose curve (75% counting efficiency). Open columns, No Ab; stippled columns, 0·1 ml anti-BSA; hatched columns, 0·3 ml anti-BSA; solid columns, 1·0 ml anti-BSA.

In cultures containing normal fresh rabbit serum, there was a 3·2-fold increase of [^{14}C]thymidine incorporation in the presence of untreated antiserum, as compared to control (no additive). The results obtained with absorbed antiserum did not differ from the control. In other cultures of the same blood donor, but in the presence of aged rabbit serum, there was no difference in thymidine uptake between cultures containing untreated antiserum, or absorbed antiserum. Both the absorbed and the untreated antiserum showed no stimulation as compared with control cultures having antigen alone or no additive at all (Table 4).

Three patients with agammaglobulinaemia, two with the sex-linked (Bruton) variety and one atypical (low γA and γM) were also studied. Their lymphocytes showed normal responses (average: 19·7; range: 3·1–42·2 fold increase) to BSA–anti-BSA complex prepared with absorbed Ab.

In order to investigate whether the stimulatory effect of the complex is in the complex precipitate itself or whether the complex is releasing some material into the medium after its interaction with complement, the complex and the normal fresh rabbit serum were incubated without cells and then centrifuged as described under 'Materials and methods'. Preliminary results (Table 5) from two experiments show that most of the stimulatory activity (about

TABLE 5. Effect of precipitate and supernate after BSA–anti-BSA complex formation

	BSA	BSA–anti-BSA complex	Complex/Ag ratio	% of activity
Unabsorbed antiserum				
Without centrifugation	355	3897	10·9	100·0
Supernate	268	2926	10·9	75·1
Precipitate	211	496	2·4	12·7
Absorbed antiserum				
Without centrifugation	355	2937	8·3	100·0
Supernate	268	2254	8·4	76·7
Precipitate	211	574	2·7	19·5

All counts are reported as dpm.

75%) is concentrated in the supernate. Virtually all of the stimulatory activity was recovered in the precipitate plus the supernate. The same results were obtained either with absorbed or with unabsorbed anti-BSA, using the same lymphocytes.

DISCUSSION

The ability of two different antigens, bovine serum albumin (BSA) and flagella of *Salmonella paratyphi* B (SPB), in the form of their specific complexes to stimulate normal human lymphocytes was demonstrated by morphologic criteria and uptake of [^{14}C]thymidine. This stimulation is highly significant when comparing [^{14}C]thymidine uptake by cultures containing the complex with control levels. When both SPB–anti-SPB and BSA–anti-BSA are taken together, paired *t*-test reveals a *P* value of $<0{\cdot}001$. The same trend was revealed by the morphological studies. The results of the microscopic examination for cell morphology can be compared with the [^{14}C]thymidine uptake studies on a qualitative basis only since these two methods measure different parameters. Not every enlarged lymphocyte produces new DNA since it has been shown (Salzman, Pellegrino & Franceschini, 1966) that morphological transformation occurs at normal rate and to the same extent under conditions in which DNA synthesis is almost completely inhibited. In addition, cells were not dividing synchronously, and the labelled thymidine was added to cultures only 24 hr before harvesting. Most studies in this experiment were evaluated by [^{14}C]thymidine uptake since it became apparent that with these stimulants this was the optimal method of assessment.

The characteristics of this response are as follows:

(a) Maximum stimulation with both complexes occurred between the 5th and the 6th day of incubation. This is approximately the same incubation period needed for maximum stimulation of sensitized lymphocytes by specific antigen (Hirschhorn *et al.*, 1963a).

(b) The presence of fresh serum appears to be necessary for the stimulation by antigen–antibody complexes, as well as for binding of antigen–antibody complexes to small lymphocytes. Uhr (1965) has shown that unlike the sensitization of macrophages, sensitization of lymphocytes by complexes is complement dependent. The effect of complement may be due to its capacity to increase the aggregation of antigen–antibody complexes (Maurer & Talmage, 1953).

However, 'aged', rather than heat-inactivated rabbit serum, was used in the experiments investigating the role of complement because in several experiments heat-inactivated rabbit serum, which had also been frozen and thawed several times, markedly stimulated lymphocytes without further additives. The reason for this observation is unclear, and further studies are needed to clarify the role of complement in this phenomenon.

Most of the BSA–anti-BSA complex stimulatory effect seems to be present in material released in the medium after its interaction with complement. This substance might be identical to what has been described as a cleavage product of C'3 with biological activity which satisfies criteria for its designation as anaphylatoxin (Dias da Silva & Lepow, 1967; Dias da Silva, Eisele & Lepow, 1967). The identification of the material must await further study.

The most likely cause for lymphocyte stimulation by complexes is a minor injury to the lymphocyte membrane brought about by non-specific attachment of the complexes to the membrane or through a stimulating factor found by interaction of the complexes with fresh serum. This injury may result in pinocytosis and the release of lysosomal enzymes, as has been shown for PHA (Hirschhorn, Hirschhorn & Weissmann, 1967) and postulated as a mechanism for rapid derepression of a resting cell with resultant cell enlargement, DNA replication and cell division. Since the cells stimulated by complex in these experiments do not respond to Ag alone, this stimulation is non-specific. The observation that complexes can stimulate cells from patients with agammaglobulinaemia, as has also been shown with PHA (Fudenberg & Hirschhorn, 1964), lends support to this concept.

A similar sequence of events at the lymphocyte membrane may be responsible for the lymphocyte response to all non-specific stimulants. Possibly even specific antigens, to which the cell donor is sensitized, act in a similar manner when they bind to a specific binding site at the cell membrane.

It is not known if this non-specific action of complexes plays a significant role *in vivo*. This kind of mechanism could result in localized lymphoid proliferation at the site of antigen–antibody interactions as occurs in serum sickness, Arthus reactions and many disease states such as rheumatoid arthritis. Such a mechanism may also underlie delayed hypersensitivity responses in which many initially non-sensitive cells accumulate at the site of interaction. Thus, small amounts of antibody formed by a few cells could bind with injected antigen forming complexes which bind to and stimulate non-sensitive lymphocytes and macrophages.

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REFERENCES

BRAY, G.H. (1960) A simple efficient liquid scintillator for counting aqueous solutions in liquid scintillation counter. *Analyt. Biochem.* **1**, 279.

BUSH, E.T. (1963) General applicability of the channels ratio method of measuring liquid scintillation counting efficiencies. *Analyt. Chem.* **35**, 1024.

COOPER, H.L. & RUBIN, A.D. (1965) RNA metabolism in lymphocytes stimulated by PHA. I. Initial response to PHA. *Blood*, **25**, 1014.

DIAS DA SILVA, W., EISELE, J.W. & LEPOW, I.H. (1967) Complement as a mediator of inflammation. III. Purification of the activity with anaphylatoxin properties generated by interaction of the first four components of complement and its identification as a cleavage product of C'3. *J. exp. Med.* **126,** 1027.

DIAS DA SILVA, W. & LEPOW, I.H. (1967) Complement as a mediator of inflammation. II. Biological properties of anaphylatoxin prepared with purified components of human complement. *J. exp. Med.* **125,** 921.

EAGLE, H. (1959) Amino acid metabolism in mammalian cell cultures. *Science,* **130,** 432.

FUDENBERG, H.H. & HIRSCHHORN, K. (1964) Agammaglobulinemia: The fundamental defect. *Science,* **145,** 611.

HIRSCHHORN, K., BACH, F., KOLODNY, R.L., FIRSCHEIN, I.L. & HASHEM, N. (1963a) Immune response and mitosis of human peripheral blood lymphocytes in vitro. *Science,* **142,** 1185.

HIRSCHHORN, R., HIRSCHHORN, K. & WEISSMANN, G. (1967) Appearance of hydrolase rich granules in human lymphocytes induced by phytohemagglutinin and antigens. *Blood,* **30,** 84.

HIRSCHHORN, K., KOLODNY, R.L., HASHEM, N. & BACH, F. (1963b) Mitogenic action of phytohemagglutinin. *Lancet,* **ii,** 305.

HIRSCHHORN, K., SCHREIBMAN, R.R., VERBO, S. & GRUSKIN, R. (1964) The action of streptolysin S on peripheral lymphocytes of normal subjects and patients with acute rheumatic fever. *Proc. nat. Acad. Sci. (Wash.),* **52,** 1151.

LING, N.R. & HUSBAND, E.M. (1964) Specific and non-specific stimulation of peripheral lymphocytes. *Lancet,* **i,** 363.

MAUER, P.H. & TALMAGE, D.W. (1953) The effect of the presence of complement in rabbit serum on the quantitative precipitin reaction. II. Effect on antigen and antibody precipitation. *J. Immunol.* **70,** 435.

MOORHEAD, J.F. & MCFARLAND, W. (1966) Efficiency of scintillation counting of tritium labelled DNA-protein in the mixed leukocyte reaction in vitro. *Nature (Lond.),* **211,** 1157.

NOWELL, P.C. (1960) Phytohemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. *Cancer Res.* **20,** 462.

RIPPS, C.S. & HIRSCHHORN, K. (1967) The production of immunoglobulins by human peripheral blood lymphocytes *in vitro. Clin. exp. Immunol.* **2,** 377.

SALZMAN, N.P., PELLEGRINO, M. & FRANCESCHINI, P. (1966) Biochemical changes in phytohemagglutinin stimulated human lymphocytes. *Exp. Cell Res.* **44,** 73.

UHR, J.W. (1965) Passive sensitization of lymphocytes and macrophages by antigen-antibody complexes. *Proc. nat. Acad. Sci. (Wash.),* **54,** 1599.