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# Effect of Anti-immunoglobulins on Rabbit Peripheral Blood Lymphocytes

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**Abstract :** The effect of anti-immunoglobulin sera on rabbit peripheral blood lymphocytes was investigated. The lymphocytes with detectable immunoglobulin on the surface showed capping, loss and regeneration of the membrane bound Ig. Following anti-immunoglobulin stimulation, transformation into blast cells was observed.

## 1. Introduction

Treatment of lymphocytes with "anti-immunoglobulin" sera can cause redistribution of the membrane bound immunoglobulin (Ig)[28] and stimulation of B cells as measured by incorporation of $^3$H-thymidine.[25] With mouse lymphocytes, the events that result in redistribution of surface immunoglobulin, following the binding of antibodies, which may or may not be sequentially related can be distinguished as "patch formation", "capping" and disappearance of the immunoglobulin from the surface.[5,16,19,28] Following anti-immunoglobulin induced loss of membrane Ig, rapid reappearance occurs. Elson *et al.*[8] have shown resynthesis to the same level, whereas other studies have demonstrated increased concentrations of the resynthesized immunoglobulin.[6,19] Stimulation of rabbit lymphocytes has been observed with anti-allotype sera,[25] anti-class specific sera[23] and antisera to Fab and Fc fragments.[10]

The discovery of easily detectable immunoglobulins on the surface of B lymphocytes,[21,22] implied that the anti-immunoglobulin induced blast transformation occurred in the B cell population. Fanger *et al.*[10] have shown that the response of rabbit peripheral blood lymphocytes to anti-immunoglobulin was not affected by treatment with antisera to rabbit thymus cells, whereas Concanavellin A (Con A) and phytohemagglutinin responses were eliminated, suggesting that B cells, and not T cells, proliferate in response to anti-immunoglobulins. Daguillard and Prochazkova[4] have correlated the degree of response of rabbit spleen cells to goat anti-rabbit immunoglobulin with the percentage of complement rosette forming lymphocytes. By autoradiography and $^3$H - thymidine uptake, Elfenbein *et al.*[9] have shown that 70% to 80% of rabbit peripheral blood lymphocytes which respond to anti-allotype antisera were complement receptor bearing. These findings suggest that only a subpopulation of B lymphocytes respond to anti-immunoglobulin.

Most immunoglobulin induced "capping", pinocytosis and regeneration of membrane bound immunoglobulin have been performed with mouse lymphocytes. In this investigation, attempts have been made to link the phenomenon of anti-immunoglobulin induced transformation of rabbit lymphocytes with membrane perturbation process such as capping and to explore the contribution, (if any), by phagocytic/adherent cells to anti-immunoglobulin mediated B cell transformation.

## 2. Materials and Methods

### 2.1. Experimental Animals

Outbred rabbits of either sex were obtained from Goodchilds Bros., Sussex, England.

### 2.2. Medium

In all the experiments "Eagles Minimal Essential Medium" containing 10% Foetal Calf Serum (FCS), penicillin (200 units/ml) and streptomycin (150$\mu$g/ml) was used.

### 2.3. Peripheral Blood Lymphocytes (PBL)

Rabbit blood obtained by ear bleeding (about 25 ml) was defibrinated by gentle shaking with glass beads (BDH, undrilled, about 25) in 20 ml. disposable "universals", immediately after bleeding and mixed with a solution of 3% gelatin in saline (2 parts of blood : 1 part of gelatin). The mixture was allowed to sediment at 37°C for 45 minutes. The supernatant which contained the lymphocytes was removed carefully and washed with warm medium (two washes, centrifugation at 200 g/10 minutes) and finally with cold medium.

### 2.4. Antisera to rabbit immunoglobulins

Two anti-rabbit immunoglobulin sera raised in goats (G101/68 and G/1286—73) were gifts from Dr. F. C. Hay, Department of Immunology, Middlesex Hospital Medical School, London.

### 2.5. $F(ab')_2$ fragment of goat anti-rabbit immunoglobulin antisera

The IgG fraction of G/101—68 antiserum prepared by DEAE-cellulose chromatography was digested with pepsin (substrate : enzyme, 20 : 1) in the presence of 0.01 M cysteine, for 12 hours at 37°C. The digest was first fractionated on Sephadex G-200, in acetate buffer, pH 4.8, 0.1 M, and the fractions of approximate molecular weight 100,000 were pooled and recycled on Sephadex G 200. The second gel filtration gave a symmetrical single peak, which on immunoelectrophoresis showed the presence of two components. The major component, $(F(ab')_2$, was of higher mobility, compared to goat IgG and the minor component was a contamination. Absorption on to DEAE-cellulose, at pH 8.1, 0.002 M phosphate buffer removed this contamination.

### 2.6. Normal Goat Immunoglobulin (NGIg)

Normal goat immunoglobulin was obtained from normal goat serum by DEAE-cellulose chromatography, at pH 8.1, 0.005 M phosphate in a batch preparation.

### 2.7. Conjugation of IgG fraction of goat anti-rabbit immunoglobulin to fluorescein

IgG fractions of G/101—68 and G/1286—73 were conjugated with fluorescein iso-thiocyanate (FITC) by the method of Johnson and Holborow.[15] Protein dissolved in bicarbonate buffered saline, pH 9.5, (20 to 25 mg/ml) was added to FITC (dry powder 20 to 40 $\mu$g FITC/mg protein) and mixed on a rotor for 1 hour at room temperature. The free fluorescein was removed by gel filtration on a Sephadex G 25 column.

### 2.8. Anti-immunoglobulin pulsing of lymphocytes and lymphocyte cultures

Lymphocytes were spun down to a pellet and incubated with IgG fraction of goat anti-rabbit IgG (G/101—68) for 1 hour at 4°C. The absolute amount of "IgG fraction of goat anti-rabbit Fc antiserum" used was given under individual experiments. In the negative controls, cells were treated with an equal amount of normal goat Ig (NGIg). After incubation the cells were washed with medium and cultured for varying time intervals at 37°C.

The pulsed lymphocytes were cultured in sterile Eagles Medium containing 10% heat inactivated FCS at 37°C in 5% $CO_2$ and 95% air in a $CO_2$ incubator (National Heinicke Company, U.S.A.). All the cultures were performed in sterile "Falcon" tubes at a cell density of $2 \times 10^6$/ml/culture.

### 2.9. Staining of lymphocytes with FITO conjugates

FITC conjugates were diluted (1 : 10 dilution) with medium containing 15mM $NaN_3$ and incubated with lymphocytes (0.1 ml diluted conjugate $2 \times 10^6$ lymphocytes) at 4°C for 1 hour. Before counting, the cells were washed at least five times with medium containing 15mM $NaN_3$.

## 3. Results

### 3.1. Specificity of the anti-immunoglobulin sera

Antiserum G/101—68 was raised in goats against rabbit Fc, but possessed reactivity against both Fab and Fc determinants. Antiserum G/1286—73 was a goat antiserum to rabbit IgG. Both antisera stained about 5% of rabbit thymocytes (G 101/68—5.6% (31/541), G 1286/73—4.9% (27/561), which most probably were B cells.

The proportion of rabbit peripheral blood lymphocytes that stained with the fluorescein conjugated G/101—68 and G/1286—73 are shown in Table I.

TABLE 1. Staining of Rabbit Peripheral Blood Lymphocytes with Fluorescein Conjugated Antisera (G101/68 and G1286/73) —Effect of Antiserum Dilution

| *Antiserum dilution* | *Percentage of cells stained* G101/68 | G1286/73 |
|---|---|---|
| 1 : 4 | 69 | 65 |
| 1 : 8 | 60 | 42 |
| 1 : 16 | 63 | 58 |
| 1 : 32 | 54 | 51 |
| 1 : 64 | 50 | 40 |

### 3.2. Capping and regeneration of membrane bound Ig

3.2.1. *Experiment :* Rabbit lymphocytes were pulsed with the IgG fraction of goat anti-rabbit IgG Fc antiserum (200 $\mu$g specific antibody/2 $\times$ $10^6$ lymphocytes) for 1 hour at 4°C. In the controls, the cells were treated with NGIg. After incubation, the cells were washed to remove excess antibody and cultured at 37°C in aliquots containing 2 $\times$ 10 lymphocytes/ml/tube for various lengths of time. Samples were removed at different time intervals, washed with medium containing 15 mM $NaN_3$ and stained with FITC—G/1286—73.

3.2.2. *Results :* Incubation of the pulsed cells at 37°C resulted in capping as determined by immunofluorescence. 100% capping of the immunoglobulin positive cells was never achieved. The highest percentage of cells with caps was observed at 45 min to 60 min at 37°C. After about 3 hours at 37°C, the number of cells with caps gradually decreased. At this time, the caps were extremely small and in some cases fluorescent "dots" were seen inside the cytoplasm of some cells. The cells treated with NGIg did not cap, but almost all the cells were patched, presumably due to the conjugated antibody used for fluorescence. The percentage of stained cells dropped to a minimum (about 20%) at 4 hours of incubation at 37°C and this correlated well with the decline of the number of capped cells (Figure 1).

### 3.3. Stimulation by IgG and F(ab')$_2$ fragment of anti-rabbit IgG antibody

Aliquots of rabbit lymphocyte suspensions in Eagles Medium (2 $\times$ $10^6$ lymphocytes/ml) were pulsed with varying quantities of IgG and F(ab')$_2$ fragment of goat anti-rabbit IgG and cultured for 48 hours to 64 hours at 37°C. After the incubation, the cells were washed with medium containing 15 mM $NaN_3$ and stained with FITC—G/1286—73. The results are expressed as the percentage of immunoglobulin positive blast cells per total immunoglobulin positive lymphocytes.

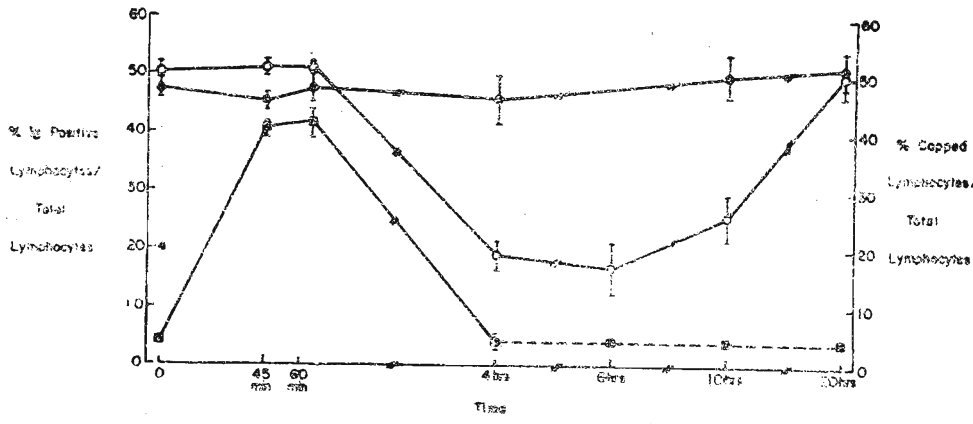


Figure 1. Anti-immunoglobulin induced redistribution of surface immunoglobulin on rabbit peripheral blood lymphocytes.

●—● $\%\ \frac{\text{Ig positive lymphocytes}}{\text{Total lymphocytes}}$ , NG Ig treated cells

o—o $\%\ \frac{\text{Ig positive lymphocytes}}{\text{Total lymphocytes}}$ , G/R IgG treated cells

■—■ $\%\ \frac{\text{Capped lymphocytes}}{\text{Total lymphocytes}}$ , G/R IgG treated cells.

Dose response curves for transformation and capping by the IgG of goat anti-rabbit IgG and for transformation by the F(ab'), of antibody are shown in Figure 2. The F(ab'), fragment of the antibody behaved in a similar fashion to IgG of antibody but the blast response was less than that of the IgG antibody (Figure 2).

### 3.4. Depletion of phagocytic/adherent cells

Rabbit PBL were depleted of phagocytic cells by carbonyl iron treatment and adherent cells by passing through a glass bead column. To lymphocyte suspensions in Eagles medium containing 10% FCS, were added a suspension of carbonyl iron ($Fe_2(CO)_9$), in medium to result a final concentration of 4 mg of carbonyl iron/$3 \times 10^6$ lymphocytes/ml and incubated at 37°C for 1 hour. These cells which had ingested the iron powder were removed together with the free carbonyl iron by two sedimentations in a strong magnetic field. The supernatants were removed, cells spun down (200 g, 10 min) washed and resuspended in medium containing 50% FCS and filtered through a glass bead column, preheated to 37°C (BDH, Glass Beads for Gas Chromatography, coated with silicone, 10 ml of glass beads/$10^8$ lymphocytes). The non-adherent cells were collected, washed and resuspended in medium containing 10% FCS. The composition of the phagocytic/adherent cells depleted lymphocytes was determined by neutral red staining.

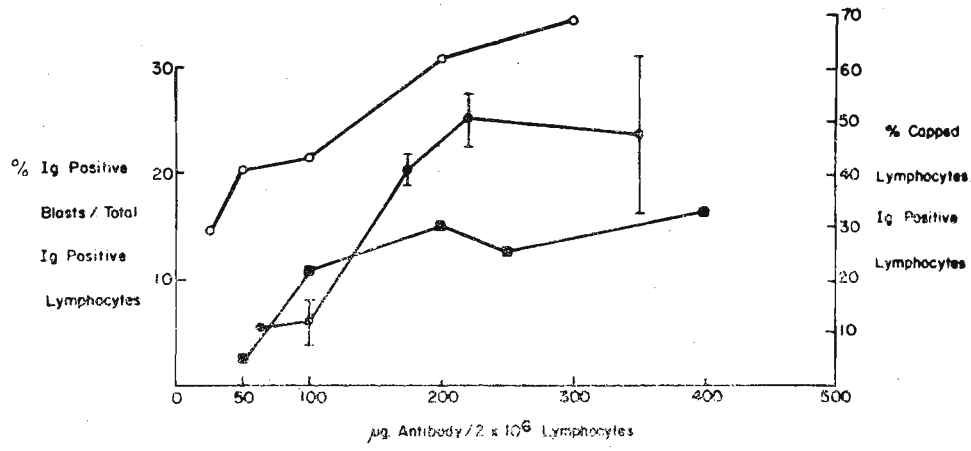


Figure 2. Dose response curves for capping and transformation of rabbit peripheral blood lymphocytes.

o — o Capping by G/R IgG, IgG antibody

●—● transformation by G/R IgG, IgG antibody.

■—■ transformation by G/R IgG, $F(ab')_2$ of antibody.

TABLE 2. Composition of the Phagocytic/Adherent Depleted Lymphocyte Population

| | | | | | | |
|---|---|---|---|---|---|---|
| No. lymphocytes, used | $3\times10^7$ | $3\times10^7$ | $7.5\times10^7$ | $4\times10^7$ | $1.35\times10^7$ | $5.6\times10^7$ |
| No. lymphocytes recovered | $1.8\times10^7$ | $1.5\times10^7$ | $4\times10^7$ | $1.9\times10^7$ | $7.5\times10^7$ | $3.6\times10^7$ |
| Percentage yield (approximate) | 60 | 50 | 53 | 47 | 55 | 64 |
| Percentage neutral red positive | 1.6 | 1.1 | 0.9 | 0.5 | 1.2 | 1.0 |

(Each column represents results of one experiment)

### 3.5. Stimulation of rabbit B lymphocytes in the absence of phagocytic/adherent cells

Untreated and adherent cells depleted cell populations were pulsed with goat anti-rabbit IgG (250 to 500 $\mu$g antibody/$2\times10^6$ lymphocytes). The negative controls were cells pulsed with NGIg. The pulsed cells were incubated at 37°C for 48 hours to 64 hours, washed with medium containing 15 mM $NaN_3$ and stained with FITC-G/ 1286—73.

Depletion of phagocytic/adherent cells reduced the blast responses to a level ntermediate between the response of negative and positive controls. (Table 3).

TABLE 3. Transformation of Phagocytic/Adherent Depleted Lymphocytes

| *Lymphocyte population* | *Pulsed with* | *Blasts/Total Ig + ve cells* | | | | | *Mean ± S.D.* |
|---|---|---|---|---|---|---|---|
| Normal L | NGIg | 12.3 (14/113) | 7.7 (6/77) | 5.4 (4/73) | 4.5 (5/111) | 7.4 (10/135) | 7.3 ± 3.0 |
| Normal L | G/R IgG | 33.7 (28/83) | 25 (33/132) | 21.6 (22/102) | 25.2 (26/103) | 19.3 (23/119) | 24.9 ± 5.5 |
| Depleted L | NGIg | 9.2 (9/97) | 3.9 (4/101) | 5.7 (6/104) | 7.2 (14/193) | 5.6 (7/125) | 6.3 ± 1.9 |
| Depleted L | G/R IgG | 22.3 (21/94) | 14.4 (43/297) | 7.6 (8/104) | 15.6 (16/102) | 11.8 (16/133) | 14.3 ± 5.4 |

L = Lymphocytes
Each vertical column represents results of one experiment.
Numbers in parenthesis represent the actual number of cells counted.
(No. immunoglobulin positive blasts/total immunoglobulin positive cells)

### 3.6. Isolation of phagocytic/adherent cells and composition of isolated cells

Lymphocyte suspensions in Eagle's medium ($10^6$ cells/ml) were incubated in disposable petri dishes (3ml/petri dish) at 37°C for 30 min. After incubation the non-adherent cells were removed by gentle washing and the adherent cells isolated with the help of the plunger of a disposable injection syringe used as a rubber policeman, the non-adherent cells were further depleted of phagocytic adherent cells by carbonyl iron treatment and glass bead columns. The composition of the isolated adherent cell population was determined by neutral red staining and acridine orange staining.

TABLE 4. Composition of isolated adherent cells

| | | | |
|---|---|---|---|
| *Untreated lymphocytes* | | | |
| %Lymphocytes | 66 (125/189) | 73.4 (94/124) | 70 (54/77) |
| %Myeloid cells | 33.8 (64/189) | 26.5 (34/128) | 29.8 (23/77) |
| *Non-adherent lymphocytes* | | | |
| %Lymphocytes | 63.5 (134/211) | 83.8 (323/385) | 97.1 (137/141) |
| %Myeloid cells | 36.4 (77/211) | 16.3 (63/385) | 2.9 (4/141) |
| *Isolated adherent cells* | | | |
| %Lymphocytes | 18.4 (26/141) | 33.1 (53/154) | 20.3 (11/54) |
| %Myeloid cells | 81.5 (115/141) | 73.7 (104/154) | 79.6 (43/54) |

(Each vertical column represents results of one experiment)
(Numbers in parenthesis represent the actual number of cells counted).

### 3.7. Stimulation of adherent/phagocytic cells depleted lymphocytes reconstituted with isolated adherent cells

The adherent cell depleted lymphocytes cell population was divided into two parts and one part was reconstituted with the isolated adherent cells. Non-depleted and depleted and reconstituted lymphocytes were pulsed with goat anti-rabbit IgG. In the negative controls, all these cell populations were pulsed with NGIg. The pulsed cells were then washed to remove the free antibody and cultured at 37°C for 48 hours to 64 hours.

Reconstitution of depleted lymphocyte preparations with isolated adherent cells partially restored the stimulation by anti-immunoglobulin. This suggests that either the adherent myeloid cells are involved in anti-immunoglobulin induced B cell stimulation or that adherent lymphocytes which were removed during depletion also contribute to B cell stimulation. The results are summarised in Table 5.

TABLE 5. Transformation of 'Phagocytic/Adherent' Depleted Lymphocytes Reconstituted with Isolated Adherent Cells.

| *Lymphocytes* | *Pulsed with* | *% Blasts/Total immunoglobulin positive cells* | | | | *Mean ±S.D* |
|---|---|---|---|---|---|---|
| Untreated L | NGIg | 8.3 (9/108) | 1.6 (2/118) | 4.6 (6/129) | 4.7 (5/106) | 4.8 ± 2.7 |
| Untreated L | G/R IgG | 27.3 (29/106) | 18.5 (21/113) | 13.7 (16/116) | 27.5 (35/127) | 21.7 ± 6.8 |
| Depleted L | NGIg | — | — | 3.7 (4/106) | 3.4 (4/117) | 3.5 ± 0.2 |
| Depleted L** | G/R IgG | 6.6 (10/151) | 6.8 (8/136) | 7.9 (10/126) | 7.6 (7/91) | 7.2 ± 0.6 |
| Depleted L * Adherent cells | NGIg | — | — | 1.7 (2/117) | 6.6 (9/135) | 4.1 ± 3.5 |
| Depleted L * Adherent cells | G/R IgG | 14.2 (16/112) | 19.8 (23/117) | 9.8 (10/102) | 22.3 (27/121) | 16.5 ± 5.6 |

(L = Lymphocytes.
( ** cf. Table 3, individual variation, 7.3—22.3)
(Each vertical column represents results of one experiment.)

## 4. Discussion

Rabbit peripheral blood has been shown to contain a higher proportion of immunoglobulin bearing cells than human or mouse blood. The average distribution is about 20% in man and 27% in mouse.[29] By the mixed antiglobulin reaction, Coombs *et al.*[3] have found wide variation in the number of rabbit lymphocytes bearing IgG, ranging between 24 and 67. Results presented in Table I demonstrate a narrower distribution (40 to 69) in the number of immunoglobulin positive lymphocytes in rabbit peripheral blood, as estimated by immunofluorescence.

The redistribution of the membrane bound immunoglobulin followed a similar pattern to those described for mouse lymphocytes.[8,28] In contrast to mouse B lymphocytes, 100% capping of the membrane bound immunoglobulin of the immunoglobulin positive cells was not achieved. Greaves and Janossay[12] have quoted a personal communication from Taylor *et al.* that rabbit (B?) lymphocytes, which are very effectively activated by anti-immunoglobulin, cap less readily than mouse immunoglobulin bearing cells.

Both capping and transformation required a minimal concentration of the anti-immunoglobulin antibody, but no stimulation (or relatively little) was observed at the minimal concentration of antibody which resulted in capping (Figure 2). Elson *et al.*[8] observed that antibody concentrations which induced cap formation did not stimulate mouse spleen cells. But an unpublished observation has been quoted that with rabbit lymphocytes, antibody concentrations which capped, also stimulated the lymphocytes.

$F(ab')_2$ fragment of the antibody behaved in a similar fashion to the IgG of the antibody. But the degree of stimulation was less than the IgG antibody (Figure 2). Fanger *et al.*[10] have observed similar behaviour for some F(ab'), preparations of goat antibody to rabbit IgG Fab and Fc.

In lectin induced transformation of lymphocytes, the removal of the bound lectin at any time upto 18 hours, by adding the appropriate competing sugar was found to diminish the response[18] suggesting that the prolonged or repetitive stimulation or contact of the stimulant is required for activation. The experiments reported here appear to contradict these findings because the excess antibody was washed away after pulsing of the lymphocytes. Figure 1 demonstrates that about 10% to 15% of the immunoglobulin positive cells did not cap, and the surface immunoglobulin (presumably with the bound antibody) persisted up to 20 hours. It would be interesting to know whether these cells that did not cap are the ones that transformed. Furthermore, cytophilic binding of the goat antibody to lymphocytes and myeloid cells could occur, and the elution of these cytophilically bound antibody at 37°C could maintain some free antibody in the culture.

All attempts to differentiate the contribution by phagocytic/adherent cells to transformation from that of cross linkage of the surface immunoglobulin were not entirely successful as all the available techniques for specificially depleting phagocytic cells possess some inadequacies. Methods like carbonyl iron treatment and adherence to glass beads do not result in a specific depletion of a given population and suffer from the disadvantage of poor recovery.

Depletion of phagocytic/adherent cells (Table 2) diminished the blast response by about half (Table 3). If the phagocytic/myeloid cells play a role in anti-immunoglobulin transformation, then one would expect to see a greater reduction in the

degree of blast transformation. The partial restoration of the response by the addition of isolated adherent cells would imply three possibilities : firstly, that some of the blast forming cells possessing adherent properties were removed during depletion, secondly, that phagocytic/adherent cells were essential for cooperation and thirdly, that both factors were contributory. Some myeloid cell populations are said to possess adherent properties compared to active adherence by macrophages and granulocytes.[26] In general, blast cells and dividing and/or antibody forming cells tend to adhere more than resting small lymphoctes.[13] Absorption of mouse spleen cell suspensions onto nylon wool columns markedly depleted complement receptor-bearing lymphocytes.[2] This taken together with the findings of Daguillard and Prochazkova[4] and Elfenbein *et al.*[9] where complement receptor-bearing cells were said to be responsible for anti-immunoglobulin mediated transformation, provide indirect evidence that the observed reduction in the blast response of the depleted populations was due to preferential removal of the potential blast forming cells. This was supported by the fact that lymphocytes in the isolated adherent cells were transformed with anti-immunoglobulin (Table 6).

TABLE 6. Transformation of Adherent Lymphocytes

| *Lymphocytes* | *Pulsed with* | *% Blasts/Total* | *Ig ± ve cells* |
|---|---|---|---|
| Untreated L | NGIg | 3.5 (4/113) | 2.1 (2/92) |
| Untreated L | G/R IgG | 21.1 (23/109) | 18.3 (18/98) |
| Non-adherent cells | G/R IgG | 5.6 (7/123) | 12.8 (13/101) |
| Non-adherent-carbonyl iron treated | G/R IgG | — | 18.0 (17/94) |
| Adherent cells | G/R IgG | 21.0 (20/95) | 23.7 (19/80) |
| Adherent cells-carbonyl iron treated | G/R IgG | 21.0 (20/95) | 14.1 (15/106) |
| Adherent cells plus carbonyl iron treated non-adherent cells | G/R IgG | 7.3 (7/95) | — |

(L = Lymphocytes)
(Each vertical column, results of one experiment)

Are any of the anti-immunoglobulin induced changes observed at cell membrane level directly responsible for stimulation ? In both instances, cross linking of the surface immunoglobulin seems to be a common feature. Fanger *et al.*[10] have shown that bivalent antibody fragments, $(F(ab')_2)$, of goat anti-rabbit IgG Fab/Fc stimulated $^3$H-thymidine uptake, whereas Fab′ fragments were ineffective in triggering the

lymphocytes, but inhibited the effect of intact goat anti-rabbit Fab. But when a rabbit antibody to the Fab of goat anti-rabbit Fab antibody was applied, incorporation of $^3$H-thymidine was induced, suggesting that cross linkage plays an important role in anti-immunoglobulin transformation of lymphocytes. Similarly, in the case of anti-allotype transformation, a second antibody to the allotypic determinants of the first anti-allotype antibody has been said to augment the response.[24]

The phenomenon of capping, *per se*, was not thought to be directly responsible for cell stimulation.[12] A linear co-polymer of glutamine, alanine and tyrosine (GAT) was bound to and induced cap formation from both GAT responder and non-responder mice.[7] Moreover, neither anti-HL-A nor anti-H-2 antisera, which induce cap formation of the corresponding receptors, stimulated lymphocyte proliferation.[17] In the case of mouse spleen cells, concentrations of anti-mouse immunoglobulin antibody which induced cap formation did not stimulate the incorporation of $^3$H-thymidine.[8]

Some evidence suggests that patch formation is more relevant to B cell triggering than capping. Soluble Concanavellin A (Con A) caps, but does not activate B lymphocytes whereas insoluble Con A does.[1] Phytohaemagglutinin (PHA) and Pokeweed Mitogen (PWM) covalently linked to Sepharose 4B particles, induced a proliferative response in normal spleen cells, similar to that observed with soluble mitogens.[11] With these insoluble mitogens, capping is physically prevented, but patch formation can still occur. When a cell is capped, presumably all the receptors are moved with the cap, then the remainder of the membrane may be in a physical and chemical state not very different from that of the resting membrane, compared to the perturbed state when the receptors are clustered into small patches all over the cell membrane. Thus the biochemical and physiological properties of the capped membrane may be the same as that of the resting membrane, but be altered in the patched membrane.[27]

Activation of lymphocytes by non-specific mitogens, and probably by antigens also, has been described as essentially a cell surface initiated phenomenon.[13] The critical triggering events have yet to be defined, but a series of biochemical changes—(enhanced uptake of ions and metabolites,[12] increased cyclic GMP and increased metabolism of phosphatidylinositol and other phospholipids[14] are said to be initiated by the mitogen induced aggregation of membrane components. Maino *et al.*[20] have presented evidence relating stimulation of enhanced phosphatidylinositol turnover by lymphocyte surface binding ligands to transformation, and supporting the hypothesis that at least in B lymphocytes, it is initiated by cross linkage of the specific membrane components. This investigation demonstrates that the membrane bound immunoglobulin of rabbit peripheral blood lymphocytes undergo redistribution as a result of cross linking by anti-immunoglobulins. A sub-population of these cells are transformed into blast cells, probably mediated via membrane perturbation.

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