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IMMUNOGLOBULIN-LIKE SURFACE MOLECULES AND THETA ANTIGEN DURING THE SPECIFIC AND NON-SPECIFIC STIMULATION OF MOUSE SPLEEN CELLS *IN VITRO*

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SUMMARY

BALB/c spleen cells were stimulated with C57B1/6 cells, keyhole limpet haemocyanin (KLH), pokeweed mitogen (PWM) or phytohaemagglutinin (PHA). By the immunofluorescence method the development of transformed cells with immunoglobulin-like receptor molecules or theta antigen was investigated. Transformed Ig-receptor cells were predominant after stimulation with KLH, followed by PWM, PHA and allogeneic cells in decreasing order. Transformed cells with theta-antigen were clearly increased after stimulation with allogeneic cells. With all systems, more transformed cells with Ig-receptors were found in the stimulated cultures than in the control cultures, indicating that all stimulants have an effect on both cell types. The implications of the results on the immunologic interpretation of results obtained by *in vitro* stimulation of lymphocytes with the different stimulants are discussed.

INTRODUCTION

Lymphoid cells of many species respond *in vitro* to a variety of substances by increased protein, RNA and DNA synthesis, by blast transformation and by multiplication. Such stimulation is induced specifically by allogeneic cells and by antigens in sensitized cells, or non-specifically by substances such as phytohaemagglutinin (PHA) and pokeweed mitogen (PWM) (Naspitz & Richter, 1968). The systems of *in vitro* lymphocyte stimulation are widely used for assessment of the immune capacity in patients with presumed immune deficiency and for detection of abnormalities of the immune system in patients with presumed immune diseases.

In mice, antibody production to certain antigens requires the co-operation of at least two distinct cell types. In transfer experiments to irradiated recipients, marrow-derived cells generate large numbers of antibody-producing cells only in the presence of thymus-derived cells (Miller & Mitchell, 1969; Playfair, 1971). Cell mediated immunity however, seems to

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be based mainly on the thymus-derived cells (Playfair, 1971). Recently markers have been recognized which are specific for thymus cells and others, probably specific for some of the bone-marrow-dependent cells. The θ-antigen is found on the surface of thymus and thymus-derived peripheral lymphoid cells in several strains of mice such as C3H, BALB/c and C57Bl/6 (Reif & Allen, 1966). Specific antibodies against θ-C3H can be produced in AKR mice. They are usually devoid of contamination with antibodies against histocompatibility antigens as the two strains share the H-2 locus (Raff, 1969; Takahashi, Carswell & Thorbecke, 1970). Immunoglobulin-like molecules have been recognized on the outer cell surface of lymphocytes of the peripheral lymphoid organs and the blood but only very rarely on thymus cells (Raff, Sternberg & Taylor, 1970; Pernis, Forni & Amante, 1970; Rabellino *et al.*, 1971). These immunoglobulin-like molecules can react with antigen (Wigzell & Mäkelä, 1970). It is probable that the cells displaying such immunoglobulin-like receptor molecules (Ig-receptors) are bone-marrow-derived thymus-independent lymphocytes (Unanue *et al.*, 1971; Bankhurst & Warner, 1971; Jones, Torrigiani & Roitt, 1971).

In a previous communication, it was demonstrated that with mouse spleen cells both θ-antigen and Ig-receptor cells are necessary for initiation of the secondary stimulation with soluble antigen *in vitro*. For the initiation of a response to allogeneic cells, only θ-cells are necessary (Vischer & Jaquet, 1972). In order to evaluate further the role of the two cell types during the *in vitro* lymphocyte stimulation, the respective numbers and the production of both cell types were estimated during the stimulatory response by the immunofluorescence method staining for the θ-antigen and the immunoglobulin-like surface receptor molecules. The results indicate a predominance of Ig-receptor cells during the secondary stimulation with soluble antigen and a predominance of cells with θ-antigen during the stimulation with allogeneic cells. However, in both systems described considerable numbers of the other cell types are stimulated at the same time.

MATERIALS AND METHODS

Mice

Specific pathogen free mice of the BALB/c and the C3H strain were obtained from Tierfarm AG, Sisseln, and of the C57Bl/6 and the AKR strain from the Institut für biologisch-medizinische Forschung AG (Füllinsdorf, Switzerland), and were maintained under standard conditions. In all experiments, female mice of initial age 5–8 weeks were used.

Cell suspensions and cultures

Cell suspension of spleen and thymus were prepared in Eagle's minimum essential medium (MEM) (Baltimore Biological Laboratory) buffered with N-2-hydroxyethyl-piperazine-N-2-ethane-sulphonic acid (HEPES; Calbiochem) as described (Vischer & Jaquet, 1972). $5–10 \times 10^6$ cells were cultured in 1 ml RPMI 1640 medium (Microbiological Associates) without serum at 37°C as described (Vischer, 1972). 5 μl pokeweed mitogen (PWM) (Grand Island Biological Co.) or 5 μl of a 1/10 dilution of phytohaemagglutinin-P (PHA) (Difco) in MEM was added to some tubes in certain experiments with BALB/c mice. Cultures from keyhole limpet haemocyanin immunized mice were stimulated with 2·5 μg of the antigen. For stimulation by allogeneic cells, equal numbers of BALB/c and C57Bl/6 cells were cultured together. Viability in cultures was around 30% as determined by dye exclusion using trypan blue on the third day and declined afterwards (Vischer, 1972). Therefore, all cultures were

terminated after 3 days. Stimulation was assessed after addition of 0·5 μCi of ^{3}H-thymidine for the last 15 hr of culture by determination of the radioactivity of the acid precipitable fraction (Vischer & Jaquet, 1972). Peak stimulation occurs on day 3 with all stimulants. As all cultures reported in this paper showed appropiate stimulation, the results of ^{3}H-thymidine incorporation are omitted.

Immunization

BALB/c mice were immunized by one intraperitoneal injection of 0·1 mg limpet haemocyanin (KLH) (Calbiochem) adsorbed on bentonite (Gallily & Garvey, 1968), and were used 4–8 weeks afterwards.

Anti-θ C3H antibodies (anti-θ) were produced in AKR mice by one intraperitoneal injection of 10^6 C3H thymus cells with 10^9 pertussis organisms followed by three further injections of the same number of cells incorporated into an equal volume of Freund's complete adjuvant (Difco) (0·25 ml each time) at 2-week intervals (Munoz, 1957). Peritoneal fluid was collected 10 days after the last injection. Normal AKR serum was collected by retro-orbital bleeding. For the production of an antiserum against mouse immunoglobulins (anti-Ig), rabbits were immunized with mouse serum fraction II (Mann Research Laboratories). On immunoelectrophoresis, the anti-Ig reacted strongly with immunoglobulins and faintly with transferrin. Normal rabbit serum came from a pool obtained from several rabbits.

Fluorescent staining

The globulin fraction of the anti-Ig obtained by precipitation with ammonium sulphate at 33% was conjugated with fluorescein isothyocyanate (Baltimore Biologicals) (Holborow & Johnson, 1967). The conjugate had a fluorescein to protein ratio of 2·3 and contained 2·8 units of precipitating antibody (Holborow & Johnson, 1967). Unrelated rabbit antibodies were conjugated in the same way. Fresh or cultured lymphoid cells were stained in suspension in the following way. The cells were washed three times in cold MEM with 1% rabbit serum. 0·1 ml cell suspension containing $2–8 \times 10^6$ cells were mixed with 0·1 ml conjugate and 0·1 ml normal rabbit serum in 10 × 75-mm glass tubes. After 20 min at 4°C the cells were washed three times in cold MEM with 3% bovine serum albumine (Merz and Dade). For indirect staining, the cells were first incubated for 20 min at 4°C with 0·1 ml normal mouse serum or an appropriate dilution of the anti-θ antibody (anti-θ) and washed as before. Anti-Ig conjugate and normal rabbit serum were then added as before. The cells were examined in suspension or smeared on slides with a cytocentrifuge (Shandon). Preliminary experiments indicated no difference between the two methods and only the second one was subsequently used. The slides were examined with a Reichert Diapan microscope fitted with an iodine quartz lamp using a dark field condensor, a specific fluorescein interference filter (Wild, Heerbrugg, Switzerland; maximal transmission at 495 nm and less than 10% transmission at 500 nm) and a GG 13 c barrier filter (Wild). As the fluorescein filter has some transmission in the red, the green fluorescence was easily seen against the red background. The cells were evaluated under immersion with a final magnification of ×480. For photographs, Ektachrome daylight high speed films was used.

Evaluation of cells

Only intact cells were evaluated. They were easily recognized under dark ground illumination by their clear cytoplasm and distinctly separate nucleus. Macrophages could be

recognized by their size, abundant cytoplasm with ingested debris, and the relatively small nucleus, and were not counted. Large cells with a diameter twice that of small lymphocytes and a large nucleus were taken as transformed. Erythrocytes were easily recognized and did not interfere with differentiation. All criteria were established by marking individual cells and checking them after staining with Giemsa. For each sample at least three slides with 600 cells in sets of 100 cells were differentiated. All slides were coded. As stimulated cultures could often be recognized by the abundance of transformed cells, in most experiments involving stimulation with KLH or allogeneic cells, tubes stimulated by PWM were included to assure impartiality. In a set of 100 cells there were not enough transformed cells to differentiate them adequately into stained and unstained cells. Therefore further sets of thirty or fifty transformed cells were differentiated in stained and unstained cells. In experiments staining for θ-antigen, the indirect method was used and the percentage of θ-bearing cells calculated as the difference between cells stained after treatment with anti-θ, and cells stained after treatment with normal mouse serum and anti-Ig. Staining directly with anti-Ig and staining after treatment with normal mouse serum gave similar results. Therefore, no mention is made in which way the results for anti-Ig staining are obtained in this paper.

Specificity of the antibodies

Anti-Ig. (1) Unrelated fluorescinate-labelled rabbit antibodies gave no staining of mouse cells with the method used.

(2) The percentage of lymphoid cells stained in fresh suspensions correspond to those reported by others (Raff, Sternberg & Taylor, 1970; Pernis, Forni & Amante, 1970; Rabellino, Colon, Grey & Unanue, 1971). Less than 0·5% of thymic cells stained for surface immunoglobulins.

(3) The staining could be markedly diminished after treatment with another unconjugated antibody against mouse immunoglobulins.

(4) Using the conjugate in a higher concentration did not give an increase in stained cells using fresh cell suspension.

Anti-θ, (1) More than 95% of thymic cells of the BALB/c, C57Bl/6 and C3H could be stained. AKR thymic cells were negative.

(2) Staining of spleen and lymphnode cells of the above strains gave results similar to those reported in the literature. (Raff, 1970).

(3) BALB/c macrophages were negative.

(4) Using the antibody in a higher concentration did not increase the number of positive cells using fresh spleen cell suspension.

All results are expressed as percent stained cells $\pm$ the standard deviation. For statistical analysis, Student's *t*-test was employed.

For autoradiographs, the slides were fixed for 10 min in absolute methanol. Kodak AR 10 stripping film was used with an exposure time of 7 days. Immediately afterwards, the slides were examined under the fluorescence microscope and evaluated. No decrease in the percentage of stained cells was found after autoradiography.

RESULTS

Staining patterns

Three patterns of staining with anti-Ig were observed on lymphocytes. The first was

similar to the one described as 'caps' by Raff (1970): the staining was limited to a small portion of the edge of the cell with little or no staining in other areas (Fig. 1a). The second pattern was an extension of the previous one. In it the staining was distributed around the edge of the lymphocyte. The third pattern was more confluent throughout the cell surface (Fig. 1b). With the indirect method of staining for θ-antigen, the second and third pattern were observed on thymic cells, which do not show Ig-receptors. There was no difference between fresh and cultured lymphocytes. Transformed cells showed mainly the first pattern when only anti-Ig was used (Fig. 1c). When transformed cells from cultures of thymic cells were stained with anti-θ, all three patterns were observed. However, the

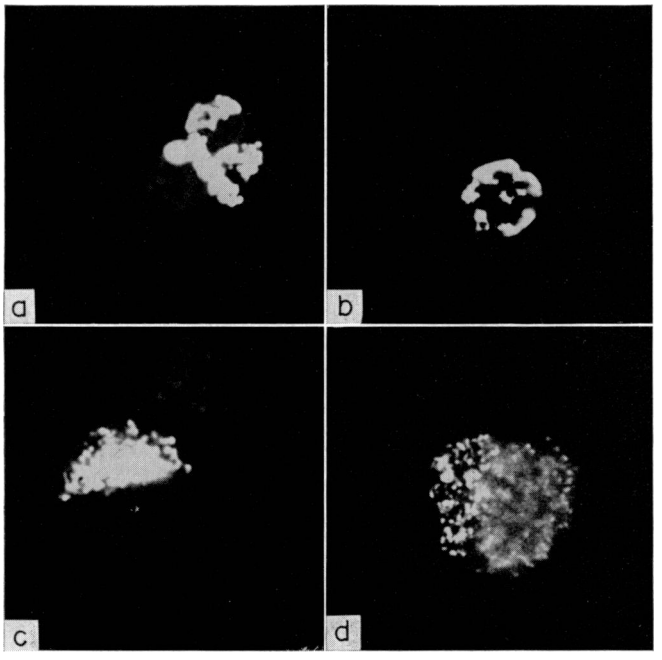

FIG. 1. Immunofluorescence of surface immunoglobulins on lymphocytes. (a) Three small lymphocytes with 'caps'; (b) small lymphocyte with the more confluent pattern; (c) transformed cell with confluent staining over half of the cell; (d) labelled transformed cell, focused on the fluorescence (magnification × 1200).

staining was often less intensive with smaller areas of the cell surface covered. In all experiments when the θ-antigen was searched for, a certain percentage of unstained cells was found, especially among the transformed cells. Therefore, BALB/c thymic cells were stimulated *in vitro* with PWM and stained for Ig-receptors and θ-antigen: No Ig-receptors were found and around 25% of the transformed cells were negative. As macrophages were not included in the differentiation, it is most likely that transformed cells have a less dense distribution of θ-antigen on their surface, thus accounting for the less intensive staining and in part for the negative cells.

Macrophages were not usually stained. Sometimes distinct fluorescent granules were observed which were not confluent and were also found after staining with unrelated antibodies. Dead cells had a dull greenish fluorescence with all conjugates.

Ig-receptors

The results of all cultures are summarized in Table 1. All unstimulated tubes contained similar percentages of cells with Ig-receptors. In stimulated tubes, the most marked increase could be observed after secondary stimulation with KLH; this increase was statistically significant when compared with the tubes stimulated with PWM or allogeneic cells. The allogeneic mixtures had the smallest percentage ($P<0{\cdot}001$). The same was observed when only the transformed cells are considered. The stimulated tubes differed clearly from each other; more than 60% of the transformed cells had Ig-receptors after secondary stimulation with KLH, against 31% after stimulation with allogeneic cells ($P<0{\cdot}001$). In tubes stimulated with PWM 50% of the transformed cells had Ig-receptors, differing thus from the two specific systems ($P<0{\cdot}001$ against tubes stimulated by allogeneic cells and $P<0{\cdot}01$ against tubes stimulated by KLH). After stimulation with PHA, 33% of the transformed cells had Ig-receptors, a value similar to the one obtained after stimulation with allogeneic cells, but different from the one obtained after stimulation with KLH ($P<0{\cdot}001$).

TABLE 1. Ig-receptors on mouse spleen cells after 3 days in culture

	Transformed cells (%)		All cells: cells with Ig-receptors (%)		Transformed cells: cells with Ig-receptors (%)	
	Unstimulated	Stimulated	Unstimulated	Stimulated	Unstimulated	Stimulated
Stimulation with allogeneic cells (BALB/c + C57B1/6) (four cultures)	3·3 ± 2·3	9·5 ± 3·3	12·5 ± 7·0	10·4 ± 3·3	54·6 ± 8·2	30·9 ± 9·0
Secondary stimulation with KLH (five cultures)	3·2 ± 1·7	12·5 ± 3·1	13·9 ± 2·7	24·2 ± 6·0	51·6 ± 5·3	63·6 ± 6·0
Non-specific stimulation with PWM (four cultures)	3·9 ± 2·0	15·2 ± 8·5	10·8 ± 2·8	17·4 ± 6·6	53·8 ± 6·5	49·8 ± 14·3
Non-specific stimulation with PHA (two cultures)	2·2 ± 1·0	29·2 ± 8·7	14·1 ± 2·7	20·2 ± 2·2	54·2 ± 11·9	32·8 ± 3·0

From the values in Table 1, the percentage transformed Ig-receptor cells relating to all cells in the tubes was calculated (Table 3). In all stimulated tubes, the percentage of transformed Ig-receptor cells was higher than in the unstimulated tubes, although the difference is relatively small after stimulation with allogeneic cells. The absolute numbers would even be higher as stimulated tubes contained more cells on day 3 than the unstimulated ones when they were counted.

θ-antigen

The experiments relating to θ-antigen are summarized in Table 2. All unstimulated

TABLE 2. Theta antigen on mouse spleen cells after 3 days in culture

	Transformed cells (%)		All cells: cells with θ-antigen (%)		Transformed cells: cells with θ-antigen (%)	
	Unstimulated	Stimulated	Unstimulated	Stimulated	Unstimulated	Stimulated
Stimulation with allogeneic cells (BALB/c + C57B1/6) (two cultures)	2·0 ± 0·9	8·7 ± 2·1	77·1 ± 6·2	82·3 ± 3·0	35·0 ± 10·1	64·3 ± 2·8
Secondary stimulation with KLH (three cultures)	3·2 ± 1·8	10·9 ± 2·9	75·2 ± 2·8	63·4 ± 4·8	30·8 ± 14·3	18·3 ± 9·3
Non-specific stimulation with PWM (two cultures)	3·2 ± 1·5	14·3 ± 2·0	76·1 ± 2·0	69·7 ± 7·7	31·8 ± 4·9	27·1 ± 20·7
Non-specific stimulation with PHA (two cultures)	2·2 ± 1·0	29·2 ± 8·7	79·0 ± 4·4	65·3 ± 2·7	25·0 ± 5·0	48·0 ± 6·1

tubes had similar percentages of θ-bearing cells. In the stimulated tubes, the highest percent of θ-bearing cells was found in the allogeneic mixtures when both the whole culture or the transformed cells only were considered ($P<0·001$ KLH; $P<0·01$ PWM). No significant difference was found between cultures stimulated with KLH or PWM. Stimulation with PHA gave results between the ones obtained after stimulation with allogeneic cells and KLH. In Table 3, the percentage transformed cells with θ-antigen relating to all cells in the

TABLE 3. Surface antigens on transformed cells*

	All cells: transformed cells with Ig-receptors (%)		All cells: transformed cells with θ-antigen (%)	
	Unstimulated	Stimulated	Unstimulated	Stimulated
Stimulation with allogeneic cells (BALB/c + C57B1/6)	1·8	2·9	0·7	6·4
Secondary stimulation with KLH	1·7	8·0	1·0	2·0
Non-specific stimulation with PWM	2·1	7·6	1·0	3·9
Non-specific stimulation with PHA	1·2	9·6	0·5	14·0

* Calculated after Table 1 and 2.

tubes was calculated from Table 2. It increased in all stimulated cultures, least after stimulation with KLH, most after stimulation with allogeneic cells.

Autoradiographs of transformed cells with Ig-receptors

In order to investigate whether the transformed cells with Ig-receptors are newly formed, 3H-thymidine (0·5 μCi) was added for the last 16 hr of culture. After the cells were stained with anti-Ig, autoradiographs were made. The results can be seen in Table 4. Over 80% of the transformed cells were labelled (Fig. 1d). More than 50% of the labelled cells had Ig-receptors after stimulation with KLH against 30% in all other tubes. This experiment indicates that the transformed cells have an active DNA metabolism.

TABLE 4. Autoradiography (^{3}H-thymidine) of cells stained in suspension for Ig-receptors after 3 days in culture (stimulation by KLH and PWM)

Transformed cells (%)	No stimulant			KLH			PWM		
	With Ig-receptors	No Ig-receptors	Total	With Ig-receptors	No Ig-receptors	Total	With Ig-receptors	No Ig-receptors	Total
Labelled	35	49	84	53	34	87	30	57	87
Not labelled	13	3	16	13	0	13	13	0	13
Total	48	52	100	66	34	100	43	57	100

In a recent paper by Hellström, Zeromski & Perlmann (1971), evidence is given that human lymphoid cells in cultures can fix L-chain determinants on their surface from the culture fluid. Fewer cells stained for L-chain determinants when a protein free medium was used for washing in contrast to a medium with added gelatine. Therefore, in one experiment, cells stimulated with PWM were stained with anti-Ig and washed with MEM and 3% bovine serum albumin as usual or with MEM without added protein. No difference was found. In another experiment, the supernatants of cultures of spleen cells stimulated with PWM or KLH for 3 days were mixed with washed stimulated thymus cultures containing many transformed cells, and were incubated for 30 min at room temperature. The thymus cells were then washed and stained with anti-Ig. No Ig-receptors were found. The same supernatants were also mixed with fresh peritoneal washings of normal BALB/c mice containing 60% macrophages and incubated for 30 min at 4°C in the presence of 10^{-4} M sodium azide, washed and stained for Ig-receptors and θ-antigen. No stained macrophages were observed.

DISCUSSION

From the last experiments, it seems unlikely that a substance released during the cultures into the fluid and then passively adsorbed on transformed cells was detected during the experiments described in this paper. Culture fluids from highly stimulated tubes containing transformed cells with Ig-determinants or θ-antigen were not capable of conferring these antigens to other cells devoid of them. The remote possibility that all factors capable of conferring passively the antigens detected to other cells were used up in the primary cultures

remains and cannot be excluded at the present time. The fact that cultured and stimulated thymic cells did not contain transformed cells with Ig-receptors suggests that the transformed Ig-receptor cells belong to the bone-marrow-derived system as well, at least in the system used. The experiments with stimulated thymus cells which were stained with anti-θ indicate that transformation reduces the density of θ-antigen on the cell surface. Peripheral thymus-derived lymphocytes already have less θ-antigen on their surface than thymocytes (Reif & Allen, 1966). Additional stimulation might reduce the amount of θ-antigen even more. Thus only part of the thymus-derived cells found in peripheral lymphoid organs might present sufficient θ-antigen to be recognized with the immunofluorescence method or with a cytotoxicity test.

The high numbers of labelled, transformed cells with Ig-receptors after incubation with ^{3}H-thymidine and staining with anti-Ig suggest that at least the transformed cells with Ig-receptors are cells newly induced by the stimulants. Any difference in the small lymphocytes could be due to a selection made by different survival of the two cell types in the cultures. In analogy, it can be assumed that the transformed cells with θ-antigen are also specifically induced. Stimulation with allogeneic cells is strongly impaired in thymectomized mice (Takiguchi, Adler & Smith, 1971), and can be inhibited by treatment of the cells with anti-θ (Vischer & Jaquet, 1972). The reaction is generally believed to represent pure cellular immunity which depends on the thymus-dependent immune system. This is consistent with the results reported here that the larger part of the transformed cells carries the θ-antigen and is thus thymus-derived. However, more transformed cells with Ig-receptors and therefore probably bone-marrow-dependent are found in tubes stimulated with allogeneic cells than in the corresponding control tubes. Two possibilities can be discussed: first, stimulation by allogeneic cells is not limited to thymus-dependent cells. During rejection of a skin-graft, antibodies are produced as well, and it is possible that also during the *in vitro* reaction some bone-marrow-derived cells are activated. Another possibility not exclusive of the first would involve the blastogenic factor described in stimulated cultures (Gordon & McLean, 1965; Spitler & Lawrence, 1969) which would induce stimulation of the Ig-receptor cells. In any case, the resulting stimulation expressed as percent transformed cells or incorporated ^{3}H-thymidine counts is due to thymus dependent and bone-marrow-dependent cells, with a predominance of the former.

Secondary stimulation with soluble antigens *in vitro* is often claimed to represent cell-mediated immunity (Mills, 1966; Oppenheim, Wolstencroft & Gell, 1967). However, correlation *in vivo* with antibody production often is as good as with delayed skin reactions, and antibody production has been demonstrated in similar systems after secondary stimulation *in vitro*, in parallel with the increased DNA synthesis (Loewi, Temple & Vischer, 1968; Benezra, Gery & Davies, 1969; Richardson & Dutton, 1964). The initiation of the reaction can be blocked by pretreatment of the cells with both anti-θ and anti-Ig in the presence of complement (Vischer & Jaquet, 1972). The experiments reported confirm the idea that secondary stimulation with antigen is a joint reaction of thymus-dependent and bone-marrow-dependent cells, with a predominance of the latter.

The results with the non-specific stimulant PWM are more difficult to interpret. PWM stimulates thymic cells very well (Vischer, unpublished results). The reaction is however only little suppressed with cells obtained from thymectomized mice (Stockman, Gallagher, Heim & Ann, 1971). It is likely that this agent stimulates various cell types and is not representative of a particular system.

PHA probably stimulates primarily only thymus-dependent cells. The reaction is suppressed when the cells are obtained from neonatally thymectomized rats (Rieke, 1966) and mice (Takiguchi, Adler & Smith, 1971; Stockman, *et. al.*, 1971). In the present experiments, a clear cut increase in transformed cells with Ig-receptors was found when compared with the unstimulated tubes. Recruiting of these cells by a blastogenic factor is the most likely explanation. From this we can conclude that blastogenic factors stimulate bone-marrow-dependent cells: whether they stimulate thymus-dependent cells as well is not known. The measured effect after stimulation with PHA, e.g. blast transformation or ^{3}H-thymidine incorporation is the result of a final stimulation of both thymus and bone-marrow-dependent cells.

Lymphocyte cultures with stimulation by different agents are widely used in man. Impaired reactivity in the mixed lymphocyte reaction or after stimulation with PHA is usually interpreted as an impairment in the thymus-dependent system. From the experiments reported it seems likely that an impairment in the bone-marrow-dependent system could be followed by a similar, if smaller reduction in certain cases.

It must be emphasized that there are differences between human peripheral lymphocytes and mouse spleen cells. Around 30% of human peripheral lymphocytes have sufficiently dense Ig-receptors to be detected easily (Papamichail, Brown & Holborow, 1971; Wilson & Nossal, 1971; Cooper, Lawton & Bockman, 1971). This is slightly less than found in mouse spleen cells. Thus the contribution of these cells to the reactions might be reduced correspondingly. That lymphocytes with dense Ig-receptors in man represent bone-marrow-derived cells is likely (Wilson & Nossal, 1971) and substantiated by their reduction or absence in some cases of agammaglobulinaemia (Papamichail, Brown & Holborow, 1971; Cooper, Lawton & Bockman, 1971). Hellström, Zeromski & Perlmann (1971) described an increase in cells staining for L-chain determinants after stimulation of human peripheral lymphocytes with PHA and allogeneic cells. This staining of transformed cells for L-chains might be similar to the results described in this report, indicating a participation of bone-marrow-dependent cells in the reaction. However, we do not know whether the staining for L-chain determinants corresponds exactly to the staining with an antibody against general immunoglobulins as used in the experiments reported here. There is evidence by indirect methods that thymus-dependent lymphocytes in mice might have L-chain determinants on their surface (Greaves, 1970; Lesley, Kettman & Dutton, 1971). In several cases of agammaglobulinaemia with no clinically apparent impairment of the cellular immune system, stimulation with PHA resulted in weak reactions when compared to cells from normal controls (Douglas, Goldberg and Fudenberg, 1970; Hosking, Fitzgerald & Simons, 1971). As other authors report a normal response (Bradley & Oppenheim, 1967), some of the discrepancies could be interpreted to be due to a variable participation of bone-marrow-dependent cells in the reaction. The results of lymphocyte cultures should be interpreted very carefully when statements about the different immune systems are made.

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