Immunology, 1967, **13**, 395.

Localization of Mouse Isoantigens on the Cell Surface as Revealed by Immunofluorescence

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(*Received* 13*th January* 1967)

Summary. An immunofluorescent technique is described which permits the localization and the semi-quantitative evaluation of isoantigens. Studies of normal and tumour cells have demonstrated that H-2 isoantigens are located in discrete areas on the cell surface. The thymus of 20–25-day-old mice was found to contain approximately 85 per cent lymphoid cells with a very low isoantigen content. These cells are considered to represent thymus cortical lymphoid cells. Cortisol treatment of mice reduced the relative number of cells with low isoantigen content to 10 per cent.

INTRODUCTION

Mouse isoantigens have been demonstrated on the cell surface of normal and tumour cells (Hellström and Möller, 1965). It has been shown that the concentration of antigenic sites may vary between cells of different tissues or at different developmental stages (Pizarro, Hoecker, Rubinstein and Ramos, 1961; Möller and Möller, 1962; Basch and Stetson, 1963; Schlesinger, 1965). Little information, however, is available on the possible quantitative variations of cellular isoantigen content among cells of the same tissue.

The immunofluorescent method first described by Coons and Kaplan (1950) possesses an advantage over other immunological techniques by allowing the detection of antigens on individual cells. When living cells are stained with isoantibody conjugates and inspected in suspension, a fluorescent ring may be seen outlining the cell surface. This 'membrane immunofluorescent reaction' introduced by Möller (1961) demonstrates the presence of isoantigens at the cell surface. Although there was often considerable variation between cells with regard to the intensity of fluorescence, the preparations studied did not allow a quantitative evaluation or a structural localization of the isoantigens.

The present work describes a technique of immunofluorescent staining of living cells followed by alcohol fixation which permits a more precise localization of isoantigens on the cell surface. The results demonstrate that isoantigenic receptors of normal and tumour cells are restricted to discrete areas on the cell surface. They have further allowed a direct microscopic differentiation of cells with high and low isoantigen content. Serological studies had indirectly shown that the thymus contains cells with lesser amounts of isoantigen (Basch and Stestson, 1962; Winn, 1962). Comparative immunofluorescent studies of thymus and lymph node cells were, therefore, carried out. It will be shown that the thymus of mice contains a large proportion of cells with a very small amount of isoantigen concentrated at a few sites on the cell surface, and that by treatment of the mice with cortisol, the relative number of these cells is drastically reduced.

MATERIALS AND METHODS

Antisera

(1) *Isoantisera.* Hyperimmune isoantisera were prepared by injecting inbred C57Bl or A/Sn mice intraperitoneally with 30×10^6 living DBA/2 P-815-X2 mastocytoma cells (Dunn and Potter, 1957), serially transplanted in ascitic form in the donor DBA/2 animals. Three to six injections were given at various time intervals, and subsequent bleedings were made from the tail to obtain serum. All isoantisera used were tested for haemagglutinating and cytotoxic activity, following the methods described by Stimpfling (1961) and by Wigzell (1965).

(2) *Heterologous antiserum.* Rat anti-mouse serum was prepared by injecting inbred Lewis rats intraperitoneally with 30×10^6 living DBA/2 mastocytoma cells. Serum was obtained 3 weeks after inoculation.

(3) *Anti-γG-immunoglobulin sera.* Rabbit anti-mouse γG-immunoglobulin (IgG) sera were prepared as follows. Rabbits were immunized by intramuscular injections of mouse IgG incorporated in complete Freund's adjuvant (Difco, Detroit). Each rabbit received two 5-mg injections of mouse IgG at 3-week intervals. Bleedings were begun 14 days after the last injection and continued for over 3 months.

Preparation of IgG

Chromatography of the crude globulin fraction from normal mouse serum, obtained by precipitation at 40 per cent saturation of ammonium sulphate, was carried out on diethylaminoethyl (DEAE)-cellulose equilibrated with 0·01 M sodium phosphate buffer, pH 7·6. As suggested by the studies of Fahey and Horbett (1959), the fraction of mouse globulin that passed directly through the DEAE-cellulose column under these conditions was found to contain only IgG and was used for immunization. This globulin preparation formed a single precipitating line in the gamma region when analysed by immunoelectrophoresis with a rabbit anti-whole mouse serum. Injection of the chromatographically purified IgG into two rabbits produced an antiserum reacting only with mouse IgG antigenic determinants (see Fig. 1).

The same method was employed to prepare IgG from rabbit anti-mouse IgG sera.

Preparation of conjugates

IgG from rabbit anti-mouse IgG serum was diluted to a final protein concentration of 10 mg/ml in an appropriate volume of 0·5 M sodium carbonate–bicarbonate buffer, pH 9·5. Fluorescein isothiocyanate (FITC) (Calbiochem, Los Angeles) was added with stirring at 4°. The fluorescein to protein weight ratio was 1:40. The pH of the reaction mixture was measured with a pH-meter during the 1st hour and adjusted to 9·5 with NaOH. The vessel was then transferred to the cold room at 4° where the reaction was allowed to continue for 18 hours with constant stirring.

Unreacted dye was eliminated by filtration of the reaction mixture through a Sephadex G–25 (Pharmacia, Uppsala) column with 0·01 M sodium phosphate buffer, pH 7·6. The labelled IgG was concentrated by pressure dialysis in the cold and fractionated on DEAE-cellulose. Stepwise elution according to Cebra and Goldstein (1965) resulted in four fractions with increasing fluorescein to protein molar ratios. The fluorescein and protein concentrations of the conjugate fractions were determined following methods

described elsewhere (Cerottini and Webb, 1957). The fourth fraction, eluted with 0·01 M sodium phosphate buffer, pH 7·6, containing 1 M NaCl, had a fluorescein to protein molar ratio of 5 and was used for the indirect immunofluorescent staining of living cells.

In some experiments direct immunofluorescent staining was carried out with mouse IgG, isolated from a hyperimmune isoantiserum by fractionation on DEAE-cellulose, and labelled with FITC as described above. After labelling the conjugate was used without subsequent DEAE-cellulose fractionation; the fluorescein to protein molar ratio was equal to 3·5. For double staining experiments the crude globulin fraction was precipitated from the rat anti-mouse serum at 40 per cent saturation of ammonium sulphate, and labelled with tetramethylrhodamine isothiocyanate (Baltimore Biological Laboratories). For this purpose, fluorescent stain was reacted with the globulin at a rhodamine

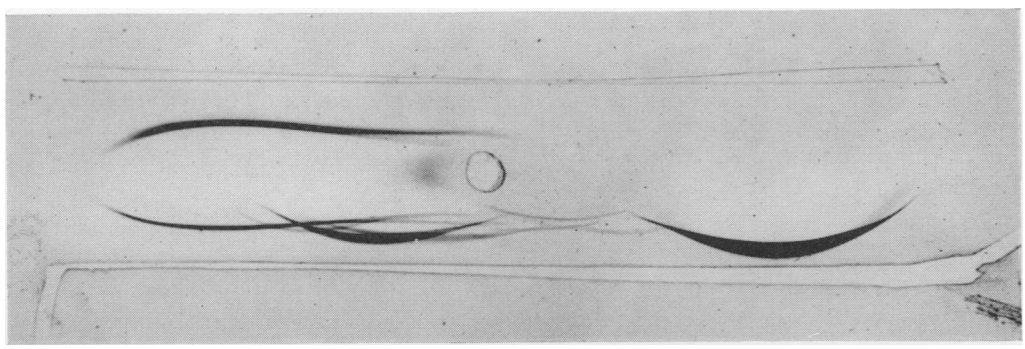


FIG. 1. Immunoelectrophoretic reaction of normal mouse serum (centre well) with rabbit IgG anti-mouse IgG (upper trough) and rabbit anti-mouse serum (lower trough). Normal mouse serum was placed in the centre well and subjected to electrophoresis in 2 per cent agar with pH 8·2 0·05 M veronal buffer. The antisera were then added to the troughs, and diffusion allowed for 12 hours at room temperature.

to protein weight ratio of 1:20. After filtration through a Sephadex G-25 column, the labelled globulin fraction was concentrated by pressure dialysis in the cold and used for immunofluorescent staining without DEAE-cellulose fractionation.

Immunofluorescent staining

Isoantisera were appropriately diluted in TD-buffer (NaCl, 8 g; KCl, 0·38 g; Na_2HPO_4, 0·1 g; Tris, 3 g; distilled water to 1000 ml; adjust pH with HCl to 7·4). Volumes of 0·1 ml of the antisera were mixed in 2 ml conical centrifuge tubes with 0·1 ml volumes of living allogeneic cells suspended in Eagle's medium without serum and adjusted to approximately 5×10^6 cells/ml. The suspensions were left at room temperature for 15 minutes with occasional shaking. The cells were then washed three times by centrifugation at 1500 rev/min and resuspension in TD-buffer with the aid of a Vortex shaker. After the final washing, the cells were suspended in 0·1 ml of the rabbit anti-mouse IgG conjugate appropriately diluted in TD-buffer, and left standing for 15 minutes at room temperature. After staining the cell suspension was washed three times with TD-buffer. The cells were re-suspended in approximately 0·5 ml of a solution of 7 per cent bovine serum albumin (BSA) in TD-buffer, mixed by pipetting with a Pasteur pipette, and centrifuged again at 1500 rev/min. The cells were re-suspended in a drop of buffered BSA,

and smears were prepared on coverslips. The smears were dried with the aid of compressed air and fixed for 5 minutes in 96 per cent ethanol. The coverslips were then placed in TD-buffer for 5 minutes, and wet mounted on a microscope slide with a drop of buffered glycerine. For semi-permanent preparations, the coverslips were sealed with nail polish. These preparations can be stored for several days at room temperature.

The slides were examined with a Wild ultraviolet microscope with a dark field condenser, using a BG 12 exciter filter and 44-50 barrier filters. The light source was an Osram HBO-200 mercury arc lamp.

Pictures were taken using Kodak Tri-X films.

RESULTS

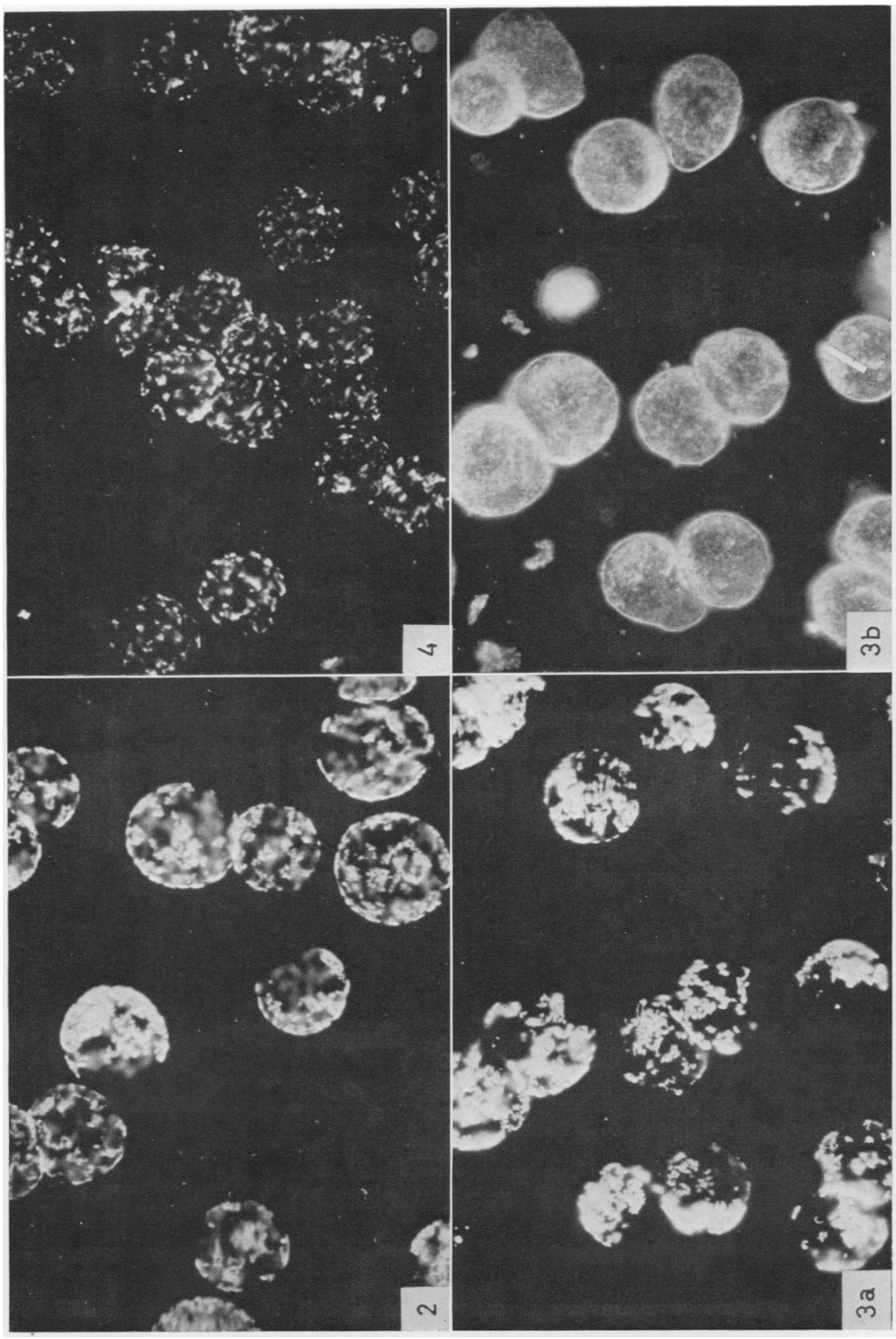
DEVELOPMENT OF THE TECHNIQUE

The coupling of a fluorochrome to protein results in a heterogeneous preparation within which the number of fluorochrome molecules per protein molecule varies markedly. Such conjugates may contain overlabelled antibody which tends to increase non-specific staining, and undercoupled antibody which may decrease the brightness of specific staining (Goldstein, Spalding and Hunt, 1962).

Previous investigations by Cebra and Goldstein (1965) have indicated that best results in indirect immunofluorescent staining of fixed cells and tissues are generally obtained by use of DEAE-cellulose column eluates with a fluorescein to protein molar ratio of 1:1. Preliminary tests showed that under the conditions of our experiments living cells do not stain non-specifically even with a heavily labelled fluorescent anti-globulin conjugate. It was, therefore, decided to fractionate rabbit anti-mouse IgG conjugate on a cellulose column and use the eluate with a fluorescein to protein ratio of 5:1, providing a very sensitive serological reagent. Control suspensions of allogeneic tumour or spleen cells first treated with normal mouse serum and, subsequently, with this highly labelled anti-globulin showed no trace of fluorescence. Similarly, syngeneic spleen cells first treated with the immune serum and then with the fluorescent antiglobulin remained unstained. On the other hand, occasional dead cells showed weak but readily visible uniform fluorescence over the entire cell surface.

When living DBA/2 mastocytoma cells were incubated first with C57Bl anti-DBA/2 hyperimmune serum and, subsequently, with the labelled rabbit anti-mouse IgG conjugate, they showed a fluorescent ring outlining the cell surface when inspected in suspension. This corresponds to the staining pattern observed in the membrane immunofluorescent reaction described by Möller (1961). Focusing up or down permitted observation of indistinct spots on the cell surface. This suggested that isoantigenic receptors were probably not uniformly distributed over the whole surface. However, cells in suspension assume a spherical shape so that microscopic resolution is poor and only a small part of the surface is in focus at a given time.

To facilitate the study of isoantigen localization on the cell surface, the living cell suspensions were first stained and then smeared on coverslips and fixed with ethanol. Care was taken to avoid cell damage during drying and fixation by suspending the stained cells in buffer containing 7 per cent BSA. Preparations thus obtained displayed well-preserved cells. The cells were flattened on the glass and their entire surface could be seen in one plane.



FIGS. 2–7. Fluorescent patterns displayed by DBA/2 normal and tumour cells. Living cell suspensions were incubated with anti-DBA/2 isoantiserum and subsequently with fluorescein-labelled rabbit anti-mouse IgG conjugate. After staining, the cells were smeared on coverslips and fixed with ethanol.

FIG. 2. Mastocytoma cells incubated with C57B1 anti-DBA/2 hyperimmune serum. Fluorescent spots and patches indicate that isoantigens are concentrated in discrete areas on the cell surface. × 450.

FIG. 3. Mastocytoma cells incubated with A/Sn anti-DBA/2 isoantiserum. (a) Similar distribution of isoantigens as seen in Fig. 2. × 450. (b) Ordinary light micro-photograph of the preparation shown in (a). The cells are morphologically intact.

FIG. 4. L 5178 Y cells incubated with A/Sn anti-DBA/2 hyperimmune serum. Similar distribution of isoantigens as observed on mastocytoma cells. × 450.

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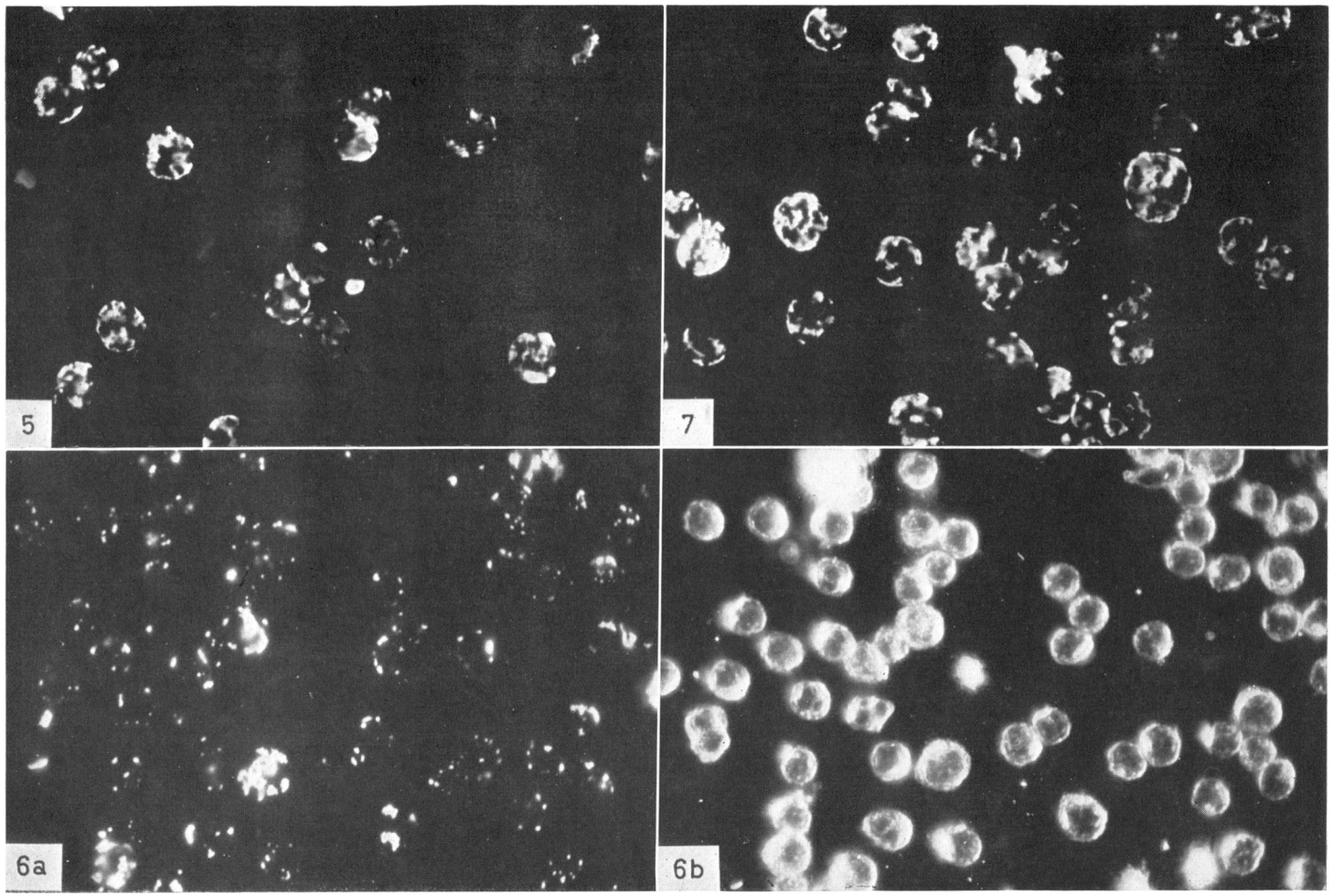

FIG. 5. Spleen cells incubated with C57B1 anti-DBA/2 hyperimmune serum. Patchy distribution of isoantigens. × 450.

FIG. 6. (a) Thymus cells of 20–25-day-old DBA/2 mice incubated with C57B1 anti-DBA/2 hyperimmune serum. The majority of the cells shows only a few tiny fluorescent spots. × 450. (b) Ordinary light micro-photograph of the preparation shown in Fig. 6(a). The identical cells which in Fig. 6(a) (ultraviolet light) show only few fluorescent spots are shown to be morphologically intact. × 450.

FIG. 7. Thymus cells of 20–25-day-old cortisol-treated DBA/2 mice incubated with C57B1 anti-DBA/2 hyperimmune serum. The majority of the cells shows a patchy fluorescence similar to spleen cells. × 450.

TUMOUR CELLS

In a first series of experiments, living DBA/2 mastocytoma cells were incubated with C57B1 anti-DBA/2 hyperimmune serum diluted to a haemugglutinating titre of 1 : 2000 to 1 : 4000. Following the indirect technique, the cells were then washed and rabbit anti-mouse IgG conjugate at a protein concentration of 1 mg/ml was added. When the ethanol-fixed cell smears were examined under the fluorescence microscope, they showed various fluorescent patterns (Fig. 2). The fluorescent stain was not uniformly distributed over the whole cell surface, but the fluorescence appeared as tiny spots or areas, clearly separated by unstained regions. In some cells the fluorescent stain was present only at one pole, resulting in a microscopic picture resembling a crescent. In other cells the stain was observed as tiny fluorescent granules or rodlets distributed over the whole cell surface or as fluorescent areas of irregular shape. When the same cells were examined by visible light microscopy, they displayed a well-preserved integrity of the cell membrane.

Control preparations of mastocytoma cells, first incubated with normal mouse serum, and then with the rabbit conjugate, remained completely unstained when inspected in the ultraviolet light. Indeed, it was necessary to examine the preparations in the visible light to ascertain the presence of cells in the microscopic field.

To rule out the possibility of an artefact introduced by the indirect immunofluorescent technique, a fluorescein-labelled IgG prepared from the C57B1 anti-DBA/2 hyper-immune serum was used in a direct test. The same results were obtained: although the staining was less bright than with the indirect method, tiny fluorescent spots often restricted to discrete areas were clearly seen.

Ethanol treatment of the cells might be held responsible for the heterogeneous fluor-escence, but it was found that cells fixed with acetone showed the same staining patterns. That the patterns observed were not due to solubilization of isoantigen by the fixation, was confirmed by the observation that stained cells fixed with ethanol and examined in suspension did not show a decrease in the intensity of fluorescence as compared to un-fixed cells.

If the unstained parts of the cell surface were not damaged, antigenic receptors, other than isoantigens, should be present in these areas. Mastocytoma cells incubated with a rhodamine-labelled globulin conjugate prepared from a rat anti-mouse serum showed fluorescent staining of the whole surface. Double staining experiments were performed with cells incubated first with the isoantiserum, then with the fluorescein-labelled rabbit anti-mouse IgG conjugate, and finally with the rhodamine-labelled rat anti-mouse con-jugate. Cells displayed green-yellow areas where both isoantibodies and heterologous antibodies were fixed, while the remaining parts of the cell surface showed red fluor-escence.

The localization of other isoantigenic receptors was also studied with an A/Sn anti-DBA/2 hyperimmune serum. This isoantiserum did not agglutinate DBA/2 erythrocytes, but was highly cytotoxic *in vitro* for mastocytoma cells in presence of complement. No striking differences were observed in the fluorescent patterns of mastocytoma cells incubated with this isoantiserum as compared to the C57B1 anti-DBA/2 serum (Fig. 3).

These experiments were repeated with the L 5178 Y tumour, a lymphoma which was originally induced in a mouse of the DBA/2 strain. The same fluorescent patterns were found, but the type of pattern seemed more uniform from one cell to another (Fig. 4).

SPLEEN, LYMPH NODE AND PERITONEAL CELLS

The C57Bl anti-DBA/2 hyperimmune serum was tested against spleen and lymph node cells from the DBA/2 mice. Suspensions of lymphoid cells were prepared as described by Brunner, Mauel and Schindler (1966) and stained by the indirect fluorescent technique. These cells displayed the same speckled fluorescent patterns as the tumour cells. Fluorescent spots were mainly found in rather large cells, while small lymphocytes showed bright dense fluorescent areas separated by narrow unstained regions (Fig. 5). DBA/2 lymphoid cells incubated with normal serum and C57Bl lymphoid cells incubated with the C57Bl anti-DBA/2 serum, which served as controls, were negative except for rare cells which were diffusely stained.

Cells obtained from the peritoneal cavity of DBA/2 mice were also tested. Staining of the cell population, mostly monocytes and lymphocytes, gave fluorescent pictures very similar to those of spleen cells. When macrophages were allowed to stick to glass, then harvested and stained, many fluorescent spots were seen on all of the cells. Control preparations incubated first with normal mouse serum and then with the rabbit anti-mouse IgG conjugate were similarly stained. This non-specific reaction was probably due to phagocytosis of the highly labelled IgG molecules and was less apparent when incubation was carried out at 4° rather than at 37°.

THYMUS CELLS

Thymus cells are known to be less sensitive to the cytotoxic effect of isoantibodies and complement and less efficient in the absorption of isoantibodies than other types of lymphoid cells. These observations may be explained either by a low isoantigen content of all thymus cells or by important variations of isoantigen content among individual cells. Experiments to test these hypotheses were performed by incubating thymus cells of 20–25-day-old DBA/2 mice with the C57Bl anti-DBA/2 hyperimmune serum, and then with the rabbit anti-mouse IgG conjugate. In contrast to the fluorescent patterns observed on spleen and lymph node cells, the great majority of the thymus cells was found to possess only two or three tiny but brilliant fluorescent spots on their surface (Fig. 6); the rest of the cells were characterized by extended areas of fluorescence similar to that observed on spleen cells. Demonstration of this important difference in isoantigen concentration was facilitated by double staining experiments. The surface of approximately 85 per cent of the cells showed a red fluorescence with a few tiny yellow spots easily distinguishable from the patchy yellow fluorescence with narrow red areas of the remaining 15 per cent of the cells. The cell population which contained smaller amounts of isoantigen was composed of cells which were somewhat smaller than small lymphocytes, although it was also possible to find rare cells of a size similar to that of large lymphocytes.

Since cortisol treatment of mice is known to induce atrophy of the thymus cortex, the effects of the drug on the lymphoid population of the thymus were studied. Twenty- to 24-day-old mice received one intraperitoneal injection of 1 mg of cortisol and were killed 3 days later. The thymuses of treated mice weighed approximately 75 per cent less than those of control animals. Immunofluorescent staining experiments demonstrated that almost all the thymus cells present in cortisol-treated mice were characterized by a high isoantigen content (Fig. 7). Among the few cells which showed only tiny fluorescent

spots, large cells were occasionally found. Microscopic counts of cells with low and high isoantigen content in normal and cortisol-treated mice are recorded in Table 1.

TABLE 1

EFFECT OF CORTISOL ON THE RELATIVE NUMBER OF THYMUS CELLS WITH VERY LOW AND HIGH ISOANTIGEN CONTENT ON THE CELL SURFACE

No. of experiment	Percentage of thymus cells with very low isoantigen content	
	Normal mice	Cortisol-treated mice
1	82	Not done
2	86	14
3	88	9

Thymus cell suspensions of 20–25-day-old normal or cortisol-treated (one intraperitoneal injection of 1 mg cortisol 3 days before killing) DBA/2 mice were prepared. The suspensions were incubated with C57Bl anti-DBA/2 hyperimmune serum, then with a fluorescein-labelled rabbit anti-mouse IgG conjugate, and finally with a rhodamine-labelled rat anti-mouse conjugate. Microscopic counts of approximately 150 cells/test suspension were carried out, separating the small or medium size lymphocytes with very low isoantigen content from similar cells with high isoantigen content.

BONE MARROW CELLS

Immunofluorescent staining of bone marrow cells showed a population which was heterogeneous both in size and in isoantigen content. Approximately 50 per cent of the large cells possessed only a few tiny fluorescent spots, while patchy fluorescent areas were seen on the surface of the remaining cells. Erythrocytes displayed very faint fluorescent spots.

DISCUSSION

The histological distribution of mouse isoantigens, particularly the H-2 isoantigens, on the cell surface of normal and tumour cells has been studied by several methods in the past. Quantitative absorption studies have demonstrated that the relative amount of isoantigen varies between different tissues (Basch and Stetson, 1962; Winn, 1962): the H-2 isoantigen concentration is higher in spleen cells than in thymus cells or in kidney cells. A correlation was found between the cytotoxic sensitivity of normal and tumour cells, and the isoantigen concentration on the cell surface (Möller and Möller, 1962; Winn, 1962); whereas all lymph node cells are susceptible to the cytotoxic effect of isoantibodies, only 10 per cent of the thymus cells are affected (Winn, 1962). Studies of complement fixation by lymph node cells and thymus cells in the presence of isoantiserum suggested that isoantigenic receptor sites are distributed in discrete patches rather than uniformly over the entire cell surface, and that the number of patches is larger for lymph node cells than for thymus cells (Winn, 1962).

The amount of H-2 isoantigen is related to the developmental stage of the mouse tissues. The H-2 isoantigen concentration is usually low in embryonic tissues, but increases during

development. Adult concentrations of H-2 isoantigen are already present in thymus cells at the 18th day of gestation (Schlesinger, 1965), whereas in the spleen full maturation of H-2 isoantigen occurs only 1–4 weeks after birth (Basch and Stetson, 1963; Möller, 1963a, b).

The methods described can only detect isoantigens at the cell population level, but quantitative variations of cellular isoantigen content among cells of the same tissue cannot be evaluated. The fact that thymus cells are less efficient in the absorption of isoantibodies than spleen cells may be due to a generally lower level of isoantigen concentration. But it is also quite possible that some thymus cells have an isoantigen content similar to that of spleen cells, while the others possess very few isoantigenic receptors.

Möller (1961) has demonstrated the presence of mouse isoantigens at the cellular level by use of the fluorescent antibody technique. Living cells were first incubated with the isoantiserum and then with a fluorescein-labelled anti-mouse IgG conjugate. The use of living cells reduces non-specific reactions to a minimum. When the stained cells were inspected in suspension they showed bright green-yellow fluorescence clearly outlining the cell surface. The effect was called the 'ring' reaction. With lymph node and bone marrow cells the ring reaction often varied in intensity, resulting in populations of cells containing a minority of weakly stained cells, but a true quantitative variation in isoantigen content could not be demonstrated.

The present studies describe the fluorescent patterns obtained by indirect staining of living cells with highly labelled rabbit IgG anti-mouse IgG followed by alcohol fixation. Normal and tumour cells stained by this technique show irregular areas of brilliant green-yellow fluorescence, clearly separated by unstained regions. The fluorescent areas contain isoantigens concentrated in tiny, often elongated granules. With the indirect method the granules have a tendency to form confluent patches of fluorescence, while direct staining results in more pronounced granularity but decreased brilliance.

A number of experiments were designed to rule out the possibility of an artefact being introduced by the techniques employed. Examination of the preparations alternatively in ultraviolet light and in ordinary light as well as with double fluorescent staining demonstrated that the cell maintained an intact membrane.

The results indicate that isoantigenic receptors of normal and tumour cells are concentrated in discrete areas of the cell surface. In the remaining parts of the cell surface isoantigens are either lacking or present in a concentration too low to be detected by the immunofluorescent method.

Mastocytoma cells, L 5718 Y cells, spleen cells, lymph node cells and peritoneal cells display essentially the same fluorescent pattern. In the thymus at least two distinct cell populations can be distinguished: the majority of the cells have a very low isoantigen content concentrated at two or three sites on the cell surface, while the remaining cells have isoantigenic receptors distibuted similarly to other lymphoid cells. Since the cortical lymphoid cells are known to make up 90 per cent of weight of the thymus (Metcalf, 1965), they presumably correspond to the cell population characterized by the low isoantigen content. The validity of this observation is confirmed by the fluorescent patterns displayed by the thymus cells of cortisol-treated mice. The important reduction in number of cells containing a very small amount of isoantigen can be correlated with the action of cortisol on the thymus. Cortisol causes a very rapid depopulation of the thymus cortex, while the medullary lymphoid cells are relatively resistant to the effect of the drug (Ishidate and Metcalf, 1963; Dougherty, Berliner, Schneebeli and Berliner, 1964). Low isoantigen

content as revealed by immunofluorescent staining may, therefore, be considered to be a marker allowing the identification of thymus cortical lymphoid cells.

In the present study no effort was made to differentiate H-2 from non-H-2 isoantigens. Further studies are being carried out to determine whether all the isoantigens are localized at the same sites or whether a given site possesses only isoantigenic acceptors with a given immunological specificity. Preliminary results indicate that the immunofluorescent patterns displayed by the mastocytoma cells after incubation either with the C57Bl anti-DBA/2 serum or with the A/Sn anti-DBA/2 serum are the same, whereas the former isoantiserum contains antibodies against more H-2 isoantigens than the latter.

ACKNOWLEDGMENTS

We are grateful to Dr H. Ginsberg and Dr N. Rose for reviewing the manuscript, and to Miss R. Bovard for excellent technical assistance. This work was supported by the Swiss National Foundation for Scientific Research.

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