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Common Antibody-induced Redistribution on Thymocytes of Strain-, Species- and Non-species-specific Antigenic Determinants Shared by Brain and Thymocytes of Mice

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Summary. Brain and thymocytes of rats, mice and fowls have certain antigenic determinants in common, which are restricted to these tissues. Among these, strain-, as well as species- and non-species-specific determinants have been detected with allotypic and heterologous antisera, respectively. With regard to the identical distribution pattern of these three different types of determinants on brain, thymus and lymph nodes it was suggested that they might belong to a common antigenic system. Using the technique of antibody-induced antigen redistribution, it is shown in the present paper that these three determinants on the thymocyte surface share a common molecular basic structure.

INTRODUCTION

Thymocytes and brain of rats, mice and fowls have some antigenic structures in common (Thiele and Stark, 1971; Golub, 1971; Thiele, Stark and Keeser, 1972; Feiglová, Pichlíková and Nouza, 1972), which are apparently lacking in other types of cells or tissues. These determinants are not uniform with respect to their antigenic specificity but can at present be classified as those which are shared by mice as well as rats, those found in all mouse strains tested so far, and those which exist in two allelic forms and which are identical with the well known Thy-1 determinants (formerly called θ) (Thiele *et al.*, 1972).

In a preceding paper (Thiele and Stark, 1974) it has been shown that anti-mouse brain serum, which contains antibodies against non-species-specific as well as against species-specific thymus–brain antigens, blocks *in vitro* the Thy-1 determinants on mouse thymocytes and brain matter, although this antiserum lacked anti-Thy-1.2 antibodies. In a reciprocal manner the anti-Thy-1.2 serum blocks the non-Thy-1.2 determinants on these tissues.

From these findings, and having regard to the identical distribution pattern of the three different determinants on brain, thymus and lymph node cells (Thiele, Stark, Keeser and Zimpel, 1974) it was suggested that they might belong to a more comprehensive basic antigenic system (Thiele *et al.*, 1972). The experiments described here, utilizing the phenomenon of antibody-induced redistribution of antigenic determinants on the cell surface, also support this assumption.

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MATERIALS AND METHODS

Cells

Suspensions of thymocytes of CBA/ASL mice 6–8 weeks old were prepared as previously described (Thiele *et al.*, 1972).

Antisera and conjugates

Rabbit anti-mouse brain sera (ABS_M) were produced as described elsewhere (Thiele *et al.*, 1972). The globulin fraction of these antisera was obtained by precipitation with 45 per cent saturated ammonium sulphate and subsequent purification on Sephadex G-25. In order to remove antibodies cross-reacting with B lymphocytes (Hudson and Phillips, 1973), the antimouse brain globulin (ABG_M) was absorbed with CBA lymph node cells (LNC) and splenic cells (SPC) before use. This was carried out by incubating with a mixture of 4×10^7 CBA LNC and SPC per millilitre for 1 hour at room temperature. The remaining thymocytolytic final titre (1 : 512) differed only slightly from that found before absorption. In some experiments an ABS_M was used, which had been found to lyse 100 per cent of mouse thymocytes as well as 100 per cent of mouse LNC. This antiserum was used without preceding absorptions.

An anti-Thy-1.2 alloantiserum was elicited by immunizing AKR/MRC mice with CBA thymocytes, following the immunizing schedule given by Reif and Allen (1966). Fluorescein isothiocyanate (FITC) conjugated globulin fractions of goat anti-rabbit globulin (FITC/GARG) were obtained from Behring-Werke/Marburg. FITC-conjugated globulin fractions of swine anti-mouse globulin (FITC/SwAMG) and tetra-ethyl rhodamine isothiocyanate (TRITC) conjugated globulin fractions of goat anti-rabbit globulin (TRITC/GARG) were obtained from Nordic Immunologic Laboratory, Tilburg.

Membrane immunofluorescence staining procedures

Isolated thymocytes were washed three times in Hanks's balanced salt solution (HBSS), pH 7·2, at room temperature. Samples of 3×10^6 cells were then resuspended in 0·05 ml of a suitable dilution of ice-cold rabbit ABG_M (1 : 30) or anti-Thy-1.2 serum (1 : 20), and incubated for 30 minutes at 0°. After three further washings at 0° in HBSS supplemented with 10 per cent foetal calf serum the cells were incubated with 0·05 ml of either TRITC/GARG or FITC/SwAMG at 0° for a further 30 minutes. Subsequently, the cells were again washed three times in ice-cold HBSS containing 10 per cent foetal calf serum. After resuspension in 0·1 ml HBSS the cells were incubated for 30 minutes at 37° to induce the capping.

For double labelling the thymocytes were treated as before. After the cells had been allowed to form caps they were quickly cooled in an ice bath to avoid endocytosis of the caps. The cells, which had been preincubated with ABG_M and TRITC/GARG were resuspended for 30 minutes in 0·05 ml of ice-cold anti-Thy-1.2 serum of the chosen dilution and—after another series of three washings—they were incubated for 30 minutes at 0° with FITC/SwAMG. In the case of preceding treatment with anti-Thy-1.2 and FITC/SwAMG counter-staining was performed first with ABG_M and then with TRITC/GARG.

After further washings (three times at 0°) the stained cells were mounted between a glass slide and a coverslip and immediately examined under a Zeiss Photomicroscope III with fluorescence equipment.

For FITC excitation filters BG 38 and KP 500 and barrier filter 53 were used. For TRITC, excitation filters BG 38 and Fl 546 and barrier filter 58.

Controls were performed by incubating thymocytes under corresponding conditions with TRITC/GARG or FITC/SwAMG only.

RESULTS

Successive treatments of CBA thymocytes with ABG_M, previously absorbed with CBA LNC and SPC and TRITC/GARG at 0°, resulted in ring-shaped membrane fluorescence (Table 1a). Subsequent warming for 30 minutes at 37° induced the development of polar caps demonstrable by red fluorescence in a high percentage of cells (up to 90 per cent) (Table 1b). Correspondingly, pretreatment of CBA thymocytes with anti-Thy-1.2 serum followed by addition of FITC/SwAMG produced green fluorescing rings and caps, respectively, in approximately the same percentages of cells (Table 1d and e; Figs 1 and 2.

TABLE 1

ANTI-MOUSE BRAIN AND ANTI-THY-1.2 SERUM-INDUCED ANTIGENIC REDISTRIBUTION ON MOUSE THYMOCYTES. SYNOPSIS OF THE VARIOUS EXPERIMENTS

(a) $(ABG_M + \text{TRITC/GARG}) \xrightarrow{0^\circ} \text{rings}^{\text{TRITC}}$.

(b) $(ABG_M + \text{TRITC/GARG}) \xrightarrow{37^\circ} \text{caps}^{\text{TRITC}}$.

(c) $(\text{TRITC/GARG}) \longrightarrow$ no fluorescence.

(d) $(\text{AThy-1.2} + \text{FITC/SwAMG}) \xrightarrow{0^\circ} \text{rings}^{\text{FITC}}$.

(e) $(\text{AThy-1.2} + \text{FITC/SwAMG}) \xrightarrow{37^\circ} \text{caps}^{\text{FITC}}$.

(f) $(\text{FITC/SwAMG}) \longrightarrow$ no fluorescence.

(g) $(ABG_M + \text{TRITC/GARG}) \xrightarrow{37^\circ} \text{caps}^{\text{TRITC}} \xrightarrow{0^\circ} + (\text{AThy-1.2} + \text{FITC/SwAMG}) \xrightarrow{0^\circ} \text{caps}^{\text{TRITC/FITC}}$.

(h) $(ABG_M + \text{TRITC/GARG}) \xrightarrow{37^\circ} \text{caps}^{\text{TRITC}} \xrightarrow{0^\circ} + (\text{FITC/SwAMG}) \xrightarrow{0^\circ} \text{caps}^{\text{TRITC}}$.

(i) $(ABG_M + \text{TRITC/GARG}) \xrightarrow{37^\circ} \text{caps}^{\text{TRITC}} \xrightarrow{0^\circ} + (ABG_M + \text{AThy-1.2} + \text{FITC/SwAMG}) \xrightarrow{0^\circ} \text{caps}^{\text{TRITC/FITC}}$.

(k) $(ABG_M + \text{TRITC/GARG}) \xrightarrow{37^\circ} \text{caps}^{\text{TRITC}} \xrightarrow{0^\circ} + (ABG_M + \text{FITC/GARG}) \xrightarrow{0^\circ}$ cell agglutination, a few $\text{caps}^{\text{TRITC/FITC}}$.

(l) $(\text{AThy-1.2} + \text{FITC/SwAMG}) \xrightarrow{37^\circ} \text{caps}^{\text{FITC}} \xrightarrow{0^\circ} + (ABG_M + \text{TRITC/GARG}) \xrightarrow{0^\circ} \text{caps}^{\text{TRITC/FITC}}$.

(m) $(\text{AThy-1.2} + \text{FITC/SwAMG}) \xrightarrow{37^\circ} \text{caps}^{\text{FITC}} \xrightarrow{0^\circ} + (\text{TRITC/GARG}) \xrightarrow{0^\circ} \text{caps}^{\text{FITC}}$.

(n) $(ABG_M + \text{TRITC/GARG}) \xrightarrow{37^\circ} \text{caps}^{\text{TRITC}} \xrightarrow{0^\circ} + (ABS_M{}^* + \text{FITC/GARG}) \xrightarrow{0^\circ} \text{caps}^{\text{TRITC/FITC}} + \text{spots}^{\text{FITC}}$.

ABG_M = anti-mouse brain globulin. ABS_M* = anti-mouse brain serum unabsorbed. AThy-1.2 = anti-Thy-1.2 serum. TRITC/GARG = rhodamine-conjugated globulin fraction of goat anti-rabbit globulin. FITC/SwAMG = fluorescein-conjugated globulin fraction of swine anti-mouse globulin. FITC/GARG = fluorescein-conjugated globulin fraction of goat anti-rabbit globulin.

Cells showing polar fluorescence did not, however, exhibit fluorescing rings or spots on the rest of the cell membrane.

Treatment of the cells with TRITC/GARG or FITC/SwAMG only, did not produce any fluorescence (Table 1c and f).

When thymocytes were first treated with rabbit ABG_M and TRITC/GARG in the cold,

then warmed and incubated again at 0° with anti-Thy-1.2 and FITC/SwAMG, caps were found to be stained with both conjugates (Table 1g). Neither red nor green fluorescence was seen in such cases except in the caps.

The same thing was observed when the experiment was carried out in reverse order, i.e. when the thymocytes were first treated with anti-Thy-1.2 and FITC/SwAMG and then with ABG_M and TRITC/GARG. In this case also the polar caps fluoresced green as well as red and no fluorescence was seen except in the caps (Table 1l; Fig. 3).

When thymocytes were treated first with ABG_M and TRITC/GARG (0°⟶37°) and then with FITC/SwAMG at 0°, only TRITC-stained caps were observed (Table 1h; Fig. 4a). Conversely, only FITC-stained caps were seen when antigen redistribution was induced by anti-Thy-1.2 serum and when the counter-staining was performed with TRITC/GARG (Table 1m; Fig. 4b). In these cases neither ring- nor spot-shaped FITC fluorescence was seen.

In another series of experiments thymocytes were incubated successively with suitable dilutions of ABG_M and TRITC/GARG at 0° followed by warming. After cooling to 0° the samples were reincubated in turn with ABG_M, anti-Thy-1.2 and FITC/SwAMG. All these latter incubations were done at 0°. Between the single incubations the cells were washed in ice-cold HBSS as described above. Even after this procedure the caps formed exhibited red as well as green fluorescence (Table 1i).

Incubation of thymocytes with ABG_M and TRITC/GARG successively at 0°, followed by warming at 37° and subsequent renewed incubation at 0° with ABG_M and FITC/GARG, resulted in agglutination of the cells, only a few of them showing observable cap formation. These caps, however, fluoresced green as well as red (Table 1k).

Experiments in which thymocytes were first processed with ABG_M and TRITC/GARG (0°⟶37°) and were then treated at 0° with an unabsorbed ABS_M batch (cytotoxic to 100 per cent thymocytes and LNC) and FITC/GARG, resulted in red and green fluorescing caps as well as in spotted green fluorescence of the rest of the cell membrane (Table 1n; Fig. 5).

DISCUSSION

In earlier experiments it has been shown that the brain and thymocytes of mice have certain antigenic determinants in common which are lacking in other cells or tissues (Thiele *et al.*, 1972; Thiele, Stark, Keeser and Zimpel, 1974).

These antigenic structures, which are demonstrable with isologous and heterologous anti-thymocyte and anti-brain sera, respectively, can at present be classified into three categories. The first of them, demonstrable by heterologous anti-brain sera, proved to be non-species-specific, and is found in thymocytes and nervous tissue of mice as well as of rats. Preliminary experiments suggest that this component is present also in human brain, although if it seems to be lacking in immature human thymocytes (Thiele *et al.*, 1973.) The second kind of determinant shared by thymocytes and brain of mice is species-specific and may also be detected by heterologous anti-brain sera (Thiele *et al.*, 1972). The third antigenic determinant present on brain and thymocytes of mice is strain-specific and identical with the well known Thy-1 determinants.

In contrast to the first two, the latter is demonstrable only with the corresponding allo-antisera.

It should be noted that the Thy-1 determinants are not identical with the above quoted species-specific structures shared by thymocytes and brain of mice. The ABG_M we used

Fig. 1

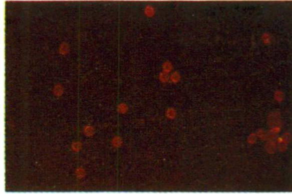


Fig. 2

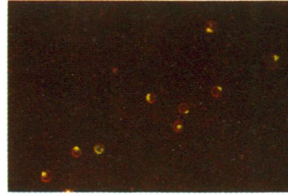


Fig. 3a

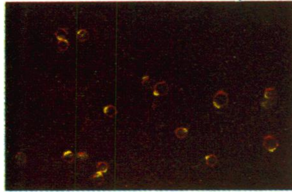


Fig. 3b

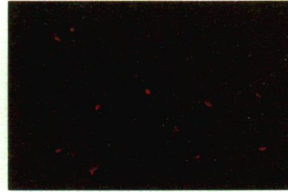


Fig. 4a

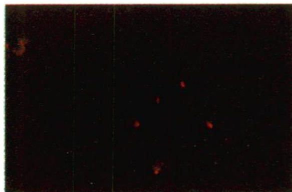


Fig. 4b

Fig. 5a

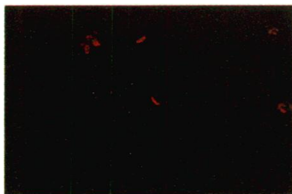
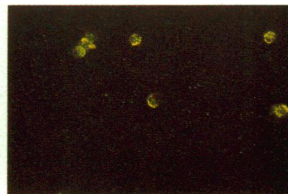


Fig. 5b



Photomicrographs of indirect immunofluorescence of living mouse thymocytes. Magnifications $\times 500$ throughout. Film: daylight Kodak high speed Ektachrome.

Fig. 1. Native unlabelled and unstained thymocytes (u.v.-illumination, excitation filters, BG 38 and KP 500, barrier filter 53) showing reddish dark field phenomenon but no fluorescence.

Fig. 2. Thymocytes treated with anti-Thy-1.2 and then FITC-conjugated swine anti-mouse globulin at 0°, subsequently warmed to 37° (30 minutes).

FITC caps when excitation filters BG 38 and KP 500 and barrier filter 53 were used. The reddish rings are the dark field effect (see Fig. 1).

Fig. 3. Thymocytes treated with anti-Thy-1.2 and then FITC-conjugated swine anti-mouse globulin at 0°, subsequently warmed to 37° (30 minutes), and, after cooling to 0°, additionally labelled with rabbit anti-mouse brain and TRITC-conjugated goat anti-rabbit globulin. (a) FITC caps when excitation filters BG 38 and KP 500 and barrier filter 53 were used. (b) TRITC caps when excitation filters BG 38 and Fl 546 and barrier filter 58 were used.

In both cases no additional specific ring or spot fluorescence was observed beyond the caps.

Fig. 4. Thymocytes treated with rabbit anti-mouse brain and then TRITC-conjugated goat anti-rabbit globulin at 0°, subsequently warmed to 37° (30 minutes), and, after cooling to 0°, additionally labelled with anti-Thy-1.2 and then FITC-conjugated swine anti-mouse globulin.

(a) TRITC caps when excitation filters BG 38 and Fl 546 and barrier filter 58 were used. (b) FITC caps when excitation filters BG 38 and KP 500 and barrier filter 53 were used. In both cases no additional specific ring or spot fluorescence is seen beyond the caps.

Fig. 5. Thymocytes treated with rabbit anti-mouse brain and then TRITC-conjugated goat anti-rabbit globulin at 0°, subsequently warmed to 37° (30 minutes), and, after cooling to 0°, additionally labelled with an unabsorbed rabbit anti-mouse brain serum* and FITC-conjugated goat anti-rabbit globulin.

(a) TRITC caps when excitation filters BG 38 and Fl 546 and barrier filter 58 were used. (b) FITC caps as well as spotted FITC fluorescence beyond the caps then excitation filters BG 38 and KP 500 and barrier filter 53 were used.

* This anti-brain serum batch was cytotoxic to 100 per cent of thymocytes as well as to 100 per cent of lymph node cells.

did not contain any anti-Thy-1.2 activities as had been shown by absorptions with AKR thymocytes and brain (Thiele *et al.*, 1972). In this context it is of interest to mention that we were unable to elicit anti-Thy-1.2 alloantisera by immunizing AKR mice with CBA brain (Thiele and Stark, unpublished data). Similar results were reported by Asakuma and Reif (1968), so we suggest that the brain-associated Thy-1 determinants may exist as haptenic structures only (Asakuma and Reif, 1968). If so, it is not surprising that these determinants might be non-immunogenic in heterologous species.

The identical distribution pattern of the different antigenic determinants on brain, thymus and lymph nodes led us to the assumption that they might belong to a more comprehensive basic system shared by brain and thymocytes (Thiele *et al.*, 1972). For this the term 'TBR-system' was proposed (Thiele *et al.*, 1974).

This suggestion was further supported by the observation of reciprocal blocking of the different determinants on brain and thymocytes by anti-brain sera of distinct specificities and by anti-Thy-1.2 alloantiserum (Thiele and Stark, 1974), which demonstrates the close proximity of the single determinants on the cell surface.

In this context the phenomenon of antibody-induced redistribution of antigenic cell surface determinants, first described by Raff, Sternberg and Taylor (1970) and re-examined since then by numerous authors (Raff, 1970; Taylor, Duffus, Raff and Petris, 1971; Kourilsky, Silvestre, Neauport-Sautes, Loosfelt and Dausset, 1972; Menne and Flad, 1973; Loor, 1973), seemed to be of interest.

The phenomenon of cap formation is apparently induced by interaction of antibody with the corresponding antigenic determinant on the cell membrane and seems to be dependent upon multivalent binding and lattice formation (Taylor *et al.*, 1971). Moreover, it is suggested that antibodies displace only the structures bearing corresponding antigenic determinants at the cell surface (Taylor *et al.*, 1971; Kourilsky *et al.*, 1972). In the light of this suggestion it should theoretically be possible to investigate molecular identities between different antigenic determinants on the cell membrane by this technique. Preud'homme, Neauport-Sautes, Piat, Silvestre and Kourilsky (1972) were able to demonstrate the molecular independence of HL-A antigens and immunoglobulin molecules at the surface of human lymphocytes in this way.

The present experiments demonstrate that polar caps induced by ABG_M comprise the determinants corresponding to the antibodies contained in this antiserum, i.e. the species- as well as the non-species-specific determinants shared by brain and thymocytes of mice. Moreover, it was found that these caps also include all the strain-specific anti-Thy-1.2 determinants, although the ABG_M used does not contain the corresponding antibodies.

The same antigenic composition of the caps was seen when they were induced with anti-Thy-1.2 serum. This indicates that the strain-, the species- and the non-species-specific determinants, which mouse brain and thymocytes have in common, are linked to a common molecular structure.

The fact that the caps were stained by ABG_M–TRITC/GARG as well as by anti-Thy-1.2–FITC/SwAMG is not contradictory to the observation of reciprocal blocking described previously (Thiele and Stark, 1974). Antigenic redistribution can be induced only with suitable antibody dilutions (Taylor *et al.*, 1971), whereas complete blocking needs antibody excess. Furthermore, the incubation times and the temperatures of the two procedures differ considerably.

However, the findings reported here seem at first glance to conflict with results recently published by Vitetta, Boyse and Uhr (1973). These authors isolated a complex containing

Thy-1 antigen from lysates of previously surface-radioiodinated mouse thymocytes by immunoprecipitation with anti-Thy-1 alloantiserum or rabbit $ABS_{Balb/c}$. They found that prior immunoprecipitation with $ABS_{Balb/c}$ removed all radioactivity that could be specifically precipitated with anti-Thy-1.2 serum, whereas prior treatment with anti-Thy-1.2 serum only partially diminished the radioactivity specifically precipitable by $ABS_{Balb/c}$. This difference between the findings of Vitetta *et al.* (1973) and the results reported here might be due to differences in the ABS_M. Hudson and Phillips (1973) recently reported that the ABG_M used by them contained not only antibodies specific for thymocytes but antibodies directed against antigenic determinants shared by thymocytes and B lymphocytes and removable by absorption with B lymphocytes.

It should be noted that Hudson and Phillips (1973) as well as Vitetta *et al.* (1973) prepared their ABS_M by the schedule described by Golub (1971), using Freund's complete adjuvant. This immunization schedule, however, in our experience, yields antisera of low antibody titre, an observation in accordance with that of Reif and Allen (1968) with regard to the elicitation of Thy-1 alloantisera.

The final thymocytolytic titre, i.e. the titre which just lyses 100 per cent of the thymocytes, in Golub's ABS_M, did not exceed a dilution of 1 : 32 (Golub, 1971).

The ABG_M used by Hudson and Phillips after absorption with liver, erythrocytes and insoluble Ig stained 100 per cent of mouse lymphocytes to a dilution of 1 : 40, when tested by indirect immunofluorescence. However, these authors did not give information on the final titre staining all thymocytes. It should certainly be taken into account that cytotoxic and immunofluorescence tests are not comparable in this regard. Vitetta *et al.* (1973) unfortunately have not made available details on the titre of their ABS_M.

A further disadvantage of Freund's complete adjuvant might be that this substance incidentally induces the development of unwanted additional antibodies. In contrast to the mentioned groups of workers, we elicited the ABS in rabbits without the addition of any adjuvant (Thiele *et al.*, 1972) and obtained ABS_M with thymocytolytic titres between 1 : 500 and 1 : 1000.

The use of antisera of high titre specificity has the advantage of allowing removal of undesired antibodies of lower titres by dilution. The ABS_M dilutions used in our previous experiments as well as those of the experiments described here did not contain any detectable antibodies reacting with B lymphocytes. Moreover, it should be noted that Vitetta *et al.* (1973), contrary to us, used undiluted ABS_M in their experiments. Nevertheless, because of the report of Hudson and Phillips (1973) the ABG_M used in the experiments described here were absorbed as a precaution with CBA–LNC and SPC. The use of unpurified LNC and SPC as a source of B lymphocytes seemed in these absorption experiments to be practicable because of the high thymocytolytic titre of the ABG_M. It was indeed found that such absorption rendered the antiserum specific for thymocytes without loss of the thymocytolytic titre. This is in accordance with the observation of Hudson and Phillips (1973), who also found that only small numbers of purified B cells were required to make their ABG_M specific for T cells.

In some experiments we used for counter-labelling an ABS_M batch which had proved to be cytotoxic to 100 per cent CBA LNC, similar to that used by Hudson and Phillips (1973). This antiserum was used unabsorbed. In these experiments fluorescing spots were observed beyond the caps. These observations suggest that the ABS_M used by Vitetta *et al.* (1973) indeed might have contained an additional 'contaminating' antibody in the above-mentioned sense. Their findings are otherwise inexplicable.

REFERENCES

ASAKUMA, R. and REIF, A. E. (1968). 'The specificity of antilymphocyte sera to thymic, splenic and leukemic lymphocytes.' *Cancer Res.*, **28,** 707.

FEIGLOVÁ, E., PICHÍLKOVÁ, L. and NOUZA, K. (1972). 'Antilymphocytic activity of rabbit anti-chicken anti-brain sera.' *Folia Biol.* (*Praha*), **18,** 256.

GOLUB, E. S. (1971). 'Brain-associated θ antigen: reactivity of rabbit anti-mouse brain with mouse lymphoid cells.' *Cell. Immunol.*, **2,** 353.

HUDSON, L. and PHILIPS, B. (1973). 'The use of column-bound B lymphocytes for absorption of antisera to T lymphocyte antigens.' *J. Immunol.*, **110,** 1663.

KOURILSKY, F. M., SILVESTRE, D., NEAUPORT-SAUTES, C., LOOSFELT, Y. and DAUSSET, J. (1972). 'Antibody-induced redistribution of HL-A antigens at the cell surface.' *Europ. J. Immunol.*, **2,** 249.

LOOR, F. (1973). 'Lymphocyte membrane particle redistribution induced by a mitogenic-capping dose of the phytohemagglutinin of *Phaseolus vulgaris*.' *Europ. J. Immunol.*, **3,** 112.

MENNE, H.-D. and FLAD, H.-D. (1973). 'Membrane dynamics of AL-A-anti-HL-A complexes of human normal and leukaemic lymphocytes.' *Clin. exp. Immunol.*, **14,** 57.

PREUD'HOMME, J. L., NEAUPORT-SAUTES, C., PIAT, S. SILVESTRE. D. and KOURILSKY, F. M. (1972). 'Independence of HL-A antigens and immunoglobulin determinants on the surface of human lymphoid cells.' *Europ. J. Immunol.*, **2,** 297.

RAFF, M. C. (1970). 'Two distinct populations of peripheral lymphocytes in mice distinguishable by immunofluorescence.' *Immunology*, **19,** 637.

RAFF, M. C., STERNBERG, M. and TAYLOR, R. B. (1970). 'Immunoglobulin determinants on the surface of mouse lymphoid cells.' *Nature* (*Lond.*), **225,** 553.

REIF, A. E. and ALLEN, J. M. (1966). 'Mouse thymic iso-antigens.' *Nature* (*Lond.*), **209,** 521.

TAYLOR, R. B., DUFFUS, W. P. H., RAFF, M. C. and PETRIS, S. DE (1971). 'Redistribution and pinocytosis of lymphocyte surface immunoglobulin molecules induced by anti-immunoglobulin antibody.' *Nature: New Biology*, **233,** 225.

THIELE, H.-G. and STARK, R. (1971). 'Antigenic relationship between thymocytes and brain tissue'. *Lancet*, **ii,** 878.

THIELE, H.-G. and STARK, R. (1974). 'Antigenic correlations between brain and thymus. II. Reciprocal blocking of antigenic determinants on mouse brain and thymocytes by antimouse brain and anti-Thy-1.2 sera.' *Z. Immun.-Forsch.*, **146,** 360.

THIELE, H.-G., STARK, R. and KEESER, D. (1972). 'Antigenic correlations between brain and thymus. I. Common antigenic structures in rat and mouse brain tissue and thymocytes.' *Europ. J. Immunol.*, **2,** 424.

THIELE, H.-G., STARK, R., KEESER, D. and ZIMPEL, H. (1973). 'Lack of antigenic correlations between brain and thymus.' *Lancet*, **ii,** 1447.

THIELE, H.-G., STARK, R., KEESER, D. and ZIMPEL, H. (1974). 'Antigenic correlations between brain and thymus. II. Further evidence for the organ specificity of the thymus-brain system in mice.' *Z. Immun.-Forsch.*, **146,** 346.

VITETTA, E. S., BOYSE, E. A. and UHR, J. W. (1973). 'Isolation and characterization of a molecular complex containing Thy-1 antigen from the surface of murine thymocytes and T cells.' *Europ. J. Immunol.*, **3,** 446.