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Surface Proteins of Thymus-Derived Lymphocytes and Bone-Marrow-Derived Lymphocytes

SELECTIVE ISOLATION OF IMMUNOGLOBULIN AND THE θ-ANTIGEN BY NON-IONIC DETERGENTS

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Accessible surface proteins of thymus-derived lymphocytes (T-cells) of normal CBA mice and bone-marrow-derived lymphocytes (B-cells) of congenitally athymic *nu/nu* mice were analysed. The surfaces of lymphocytes were radioiodinated by using the enzyme lactoperoxidase (EC 1.11.1.7), then solubilized either in acid–urea or in the non-ionic detergent Nonidet P-40. These lysates were then precipitated with antisera specific to either immunoglobulin or the θ-alloantigen in order to assess the presence of these surface markers. Comparable amounts of radioactivity in proteins specifically precipitable as immunoglobulin were obtained from T-lymphocytes and B-lymphocytes when the cells were disrupted by acid–urea. This immunoglobulin had mol. wt. approx. 180000 and was composed of light chains and μ-type heavy chains. When radioiodinated lymphocytes were solubilized with Nonidet P-40, 3–4% of radioiodinated high-molecular-weight protein of B-cells consisted of immunoglobulin, a result similar to that found with acid–urea extraction. However, with the detergent extraction, only 0.1% of T-cell surface protein was precipitable by anti-globulin reagents. The θ-alloantigen was isolated from CBA T-cells both by acid–urea and by detergent lysis. This protein possessed a mobility on polyacrylamide-gel electrophoresis in sodium dodecyl sulphate which was consistent with a mol. wt. of 60000. An identical component was isolated from the θ-positive thymoma WEHI 105. The θ-antigen was not isolated from B-cells by either of the extraction procedures used. These results provide further evidence that the surface membranes of normal T-cells and B-cells differ in physicochemical properties. In particular, various surface components possess differential solubilities in non-ionic or organic solvents. This observation provides an explanation for discrepant results that have appeared in the literature concerning the isolation of immunoglobulin from T-lymphocytes.

Immunological (Raff, 1971) and ultrastructural (Mandel, 1972; Santer *et al.*, 1973) studies have established that substantial differences exist between the outer membranes of thymus-derived lymphocytes (T-cells) and bone-marrow-derived lymphocytes (B-cells). It is conceivable that these membrane differences contribute significantly to the biological differences between these two lymphocyte types in their respective susceptibilities to activation by mitogens (Anderson *et al.*, 1972; Greaves & Janossy, 1972) and antigens (Greaves & Janossy, 1972). Further, these differences in membrane organization may play a major role in resolving the apparent contradictions when various groups have attempted to study immunoglobulins on T-cells by applying methods derived for use with B-cells (Crone *et al.*, 1972).

In the present paper, we report the results of a systematic study designed to assess the efficiency of lactoperoxidase (EC 1.11.1.7)-catalysed radioiodination coupled with various extraction techniques in the isolation and identification of two surface proteins, namely immunoglobulin and the θ-alloantigen, from thymus cells and B-cells. We provide evidence that profound differences exist between the membranes of these two lymphocyte types with respect to the presence and accessibility of these surface markers and the degree to which these components may be isolated by extraction with the non-ionic detergent Nonidet P-40.

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Materials and Methods

Cell sources and preparation

CBA/H/WEHI mice (5–7 weeks old) were used as a source of normal thymus cells. The spleens of congenitally athymic mice were used as a source of normal bone-marrow-derived (B) lymphocytes. The congenitally athymic mice were mice homozygous for the mutation 'nude' (*nu/nu*) (Flanagan, 1966), which have been derived from heterozygous parents that are in the process of being back-crossed to CBA/H/WEHI mice, but are not yet fully inbred. The *nu/nu* mice lack thymocytes (Raff, 1971; De Sousa *et al.*, 1969) and thymus-derived lymphocytes.

Single cell suspensions were prepared by teasing the organs through a fine steel mesh into chilled phosphate-buffered saline, pH 7.2 (0.05 M-sodium phosphate–0.15 M-NaCl). Cell debris and clumps were removed by settling the cell suspensions over 1 ml of foetal calf serum (Commonwealth Serum Laboratories, Melbourne, Australia) for 5 min at 4°C. After one wash with phosphate-buffered saline, erythrocytes were removed by resuspending cell pellets in 3 vol. of distilled water followed by agitation for 10s. Phosphate-buffered saline (5–10 ml) was then added and the cells were washed again, resuspended in 5 ml of phosphate-buffered saline and dead cells were removed by centrifugation through 34% (w/v) Ficoll (Pharmacia Fine Chemicals Inc., Uppsala, Sweden) as previously described (Marchalonis *et al.*, 1971). Cells prepared in this fashion were 93–98% viable as judged by Eosin dye exclusion.

Radioiodination of cell-surface proteins

External surface proteins were iodinated with carrier-free [^{125}I]iodide (90–160 mCi/ml; The Radiochemical Centre, Amersham, Bucks., U.K.) by lactoperoxidase-catalysed radioiodination (Marchalonis *et al.*, 1971).

Cells (3×10^7–1×10^8) were iodinated in batches of 5×10^6–1×10^7 cells suspended in 50 μl of phosphate-buffered saline containing 20 μg of enzyme. [^{125}I]-Iodide (100–200 μCi) was added per tube followed by 10 μl of 0.03% H_2O_2. The cells were vigorously mixed and incubated at 30°C for 5 min. Iodination was performed in 10 ml conical polystyrene centrifuge tubes and the reaction was stopped by the addition of 10 ml of chilled phosphate-buffered saline. The cells were centrifuged at 1500 rev./min for 10 min at 4°C and washed twice more with phosphate-buffered saline. Radioactivity was determined by using a Packard Autogamma Spectrometer with an NaI crystal detector.

Fractionation of labelled cell-surface proteins

Radioiodinated cells were disrupted with 10 M-urea–1.5 M-acetic acid (Marchalonis *et al.*, 1971), 1 ml of acid–urea was added per 1×10^7–2×10^7 cells. The cells were vigorously agitated and incubated at 37°C for 1–2 h. After incubation the lysate was centrifuged at 1500 rev./min at 4°C for 15 min and the supernatant retained. Acid–urea-soluble proteins were dialysed at 4°C against Tris–NaCl (0.05 M-Tris–HCl–0.15 M-NaCl) buffer, pH 8.0, overnight with two buffer changes. Alternatively, 5×10^7–1×10^8 cells were mixed with 1–2 ml of 0.5% (v/v) Nonidet P-40 (lot no. 56009; BDH, Melbourne, Australia) and incubated for 10 min at room temperature (Baur *et al.*, 1971). The cells were then centrifuged at 1500 rev./min for 10 min at 4°C and the supernatants retained. The detergent-soluble supernatants were similarly dialysed against Tris–NaCl before further analysis.

Disc electrophoresis in polyacrylamide gels

Radioiodinated cell-surface proteins or specific coprecipitates were resolved by polyacrylamide-gel electrophoresis in a discontinuous buffer system by the method of Laemmli (1970). Samples were prepared for electrophoresis by dissolving cells or precipitates in 100–300 μl of a buffer containing 10% (v/v) glycerol–2% (v/v) mercaptoethanol–3% (w/v) sodium dodecyl sulphate in 0.125 M-Tris–HCl, pH 6.8, which was 6 M in urea. The samples were heated for 5 min at 100°C and after reduction were alkylated by addition of 3–4 mg of iodoacetamide. Samples to be subjected to electrophoresis without reduction were dissolved in a similar buffer minus the mercaptoethanol. The gels were sliced into 35–40 fractions by using a Canalco slicer (Canalco, Rockville, Md., U.S.A.), and slices were counted for radioactivity in plastic tubes with the Packard Autogamma Spectrometer. Mobilities are expressed as distance migrated relative to that of a Bromophenol Blue dye marker. Because mobilities were not always identical if electrophoretic separations were not carried out at the same time, immunoglobulin marker proteins were included in each experiment to facilitate the identification of light chains and γ- and μ-heavy chains.

Identification and isolation of immunoglobulins

Immediately before co-precipitation, cell-surface proteins were centrifuged at 20000*g* for 15 min. The supernatants were retained for use in subsequent studies.

Radioiodinated cell-surface immunoglobulin was isolated by specific co-precipitation of cell-surface proteins with purified mouse IgG* and rabbit antiserum to mouse immunoglobulin (Marchalonis *et al.*, 1972). Alternatively cell-surface proteins were

* Abbreviation: IgG, immunoglobulin G.

precipitated with rabbit antiserum to mouse IgG and goat antiserum to rabbit γ-globulin (Commonwealth Serum Laboratories, Melbourne, Australia; lot no. 978–1). Controls for non-specific precipitation included precipitation of radioiodinated murine-lymphocyte cell-surface proteins with fowl IgG and rabbit antiserum to fowl IgG or normal rabbit serum and goat antiserum to rabbit IgG. In addition the amount of radioactive material that stuck to the tubes was determined by mixing radioactive cell-surface proteins with mouse IgG carrier and normal rabbit serum. Conditions for co-precipitation were determined by precipitation of trace amounts of [^{125}I]iodide-labelled mouse IgG, fowl IgG or rabbit IgG with various dilutions of carrier and antiserum, such that more than 80% of the carrier was precipitated. Generally, 100 μl of radioactive cell-surface protein was mixed with 50 μl of carrier (5 μg) and 50 μl of antiserum in 5 cm × 0.5 cm round-bottom plastic tubes that had been previously coated with 1% normal rabbit serum (by filling them with 1% normal rabbit serum and incubating 20h at 37°C and washing). Then co-precipitation mixture was incubated for 2h at 37°C and overnight at 4°C. The precipitates were resuspended by mixing and centrifuged at 20000*g* for 15min at 4°C. After the first centrifugation the supernatants were discarded and the radioactivity in the precipitates was determined. The precipitates were then resuspended in 300–400 μl of phosphate-buffered saline, transferred to fresh plastic tubes by using a separate Pasteur pipette for each transfer, and centrifuged. The precipitates were washed in this manner until the controls for non-specific precipitation had radioactivity near background values (approx. 50–100c.p.m.). At least five replicates for specific and non-specific precipitation were done in all experiments.

Identification of θ-antigen

Radioiodinated cell-surface proteins obtained from normal CBA thymus cells were co-precipitated with mouse IgG and rabbit antiserum to mouse immunoglobulin in order to remove immunoglobulin. The immunoglobulin-depleted supernatants were retained and mixed with 25 μl of a 1:10 dilution of antiserum to θ (C_3H-strain type) produced by AKR-strain mice or normal serum of strain AKR mice followed by rabbit-antiserum to mouse immunoglobulin. The anti-θ-antiserum was the generous gift of Dr. Hermann Wagner, The Walter and Eliza Hall Institute of Medical Research. The specificity tests of this antiserum have been described (Atwell *et al.*, 1973).

Additional experiments were performed with an antiserum to θ-antigen of strain C_3H produced in mice of strain AKR kindly provided by Dr. Jonathan Sprent, Basel Institute for Immunology, Basel, Switzerland. Samples of this antiserum were absorbed with AKR- or CBA-strain brain tissue as follows. A 1:100 dilution of the antiserum (1 ml) was mixed with packed brain homogenate (0.5 ml), and the mixture was gently agitated for 2h at 24°C and then centrifuged at 22000*g* for 30min. The supernatant was then used as described above, except that 250 μl was used.

Results

Isolation of lymphocyte cell-surface immunoglobulin by extraction of cell-surface proteins with urea–acetic acid

To compare the amount of radioiodinated surface immunoglobulin obtained from thymus or B (*nu/nu* spleen) lymphocytes by extraction with urea–acetic acid, 5×10^7 cells of each type were radioactively labelled with 1mCi of [^{125}I]iodide. As a routine, both cell types incorporated 25–40% of the radioactive iodide, assuming a counting efficiency of 50%. A decreased iodination efficiency was observed if the number of cells in the reaction mixture exceeded 10^7 or if the amount of lactoperoxidase and H_2O_2 was increased beyond those described in the Materials and Methods section. Some 93% of [^{125}I]iodide-labelled thymus lymphocyte surface proteins and 95% of *nu/nu* spleen cell-surface proteins were soluble in 4 ml of urea–acetic acid after incubation for 2h at 37°C. Shorter periods of incubation in this solvent released less labelled material from the cells. Exhaustive dialysis against the Tris–NaCl buffer removed 88 and 86% of the radioactivity in ^{125}I-labelled surface material from thymus and *nu/nu* spleen cells respectively. After dialysis the non-diffusible labelled material was centrifuged at 20000*g* for 15min. Approx. 40% of the high-molecular-weight surface proteins obtained from both cell types were insoluble in aqueous medium; however, 100% of the Tris–NaCl-soluble proteins were precipitated by cold 10% trichloroacetic acid.

Immunoglobulin was detected in the Tris-soluble protein mixtures by specific co-precipitation of 100 μl fractions with mouse IgG plus rabbit antiserum to mouse immunoglobulin. Table 1 shows that, after five washes, approx. 1.5% of the ^{125}I-labelled cell-surface proteins obtained from thymus or *nu/nu* spleen were specifically precipitated. When supernatants obtained from the first centrifugation of the precipitates were precipitated again with mouse IgG plus antiserum as above, no further specific precipitation was observed. However, specific precipitation of cell-surface immunoglobulin was observed when supernatants obtained from control precipitation systems were reprecipitated.

Table 1. *Isolation of surface immunoglobulin from thymus and nu/nu spleen lymphocytes by urea–acetic acid extraction*

Cells (5×10^7) were labelled with 1 mCi of [^{125}I]iodide and were lysed with urea–acetic acid. A 200 μl portion of ^{125}I-labelled surface protein soluble in Tris–NaCl was co-precipitated with rabbit antiserum to mouse IgG and mouse IgG or rabbit antiserum to fowl IgG and fowl IgG. Results represent the S.E.M. of five replicates of radioactivity precipitated after four washes.

	Radioactivity (c.p.m.)						
						Precipitated by:	
Cells	Total ^{125}I incorporation	Soluble in urea–acetic acid	Non-diffusible	Non-diffusible, soluble in Tris–NaCl buffer	Input for precipitation	Mouse IgG+ rabbit antiserum	Fowl IgG+ rabbit antiserum
CBA thymus	1.5×10^8	1.3×10^8	1.9×10^7	1.1×10^6	2.7×10^5	$4.5 \times 10^3 \pm 0.8 \times 10^3$	$7.6 \times 10^2 \pm 0.2 \times 10^2$
nu/nu spleen	1.5×10^8	1.4×10^8	2.4×10^7	1.4×10^6	3.5×10^5	$4.8 \times 10^3 \pm 0.5 \times 10^3$	$1.2 \times 10^3 \pm 0.3 \times 10^3$

Table 2. *Isolation of surface immunoglobulin from thymus with or without parathymic lymph-node cells*

Cells (5×10^7) were labelled with 1 mCi of [^{125}I]iodide and were lysed with urea–acetic acid. A 200 μl portion of ^{125}I-labelled surface proteins soluble in Tris–NaCl was co-precipitated with rabbit antiserum to mouse immunoglobulin + mouse IgG or rabbit antiserum to fowl IgG+fowl IgG. Results represent the radioactivity precipitated after four washes.

	Radioactivity (c.p.m.)						
						Precipitated with	
Cells	Total incorporation (5×10^7 cells)	Soluble in acid–urea	Non-diffusible	Soluble in Tris–NaCl	Input for co-precipitation	Mouse IgG+rabbit antiserum to mouse IgG	Fowl IgG+rabbit antiserum to fowl IgG
Thymus+parathymic lymph node	7.65×10^7	7.6×10^7	7.2×10^6	4.5×10^6	3.5×10^5	1.3×10^4	3.8×10^3
Thymus−parathymic lymph node	1.14×10^8	1.0×10^8	1.3×10^7	6.7×10^6	5.3×10^5	1.8×10^4	4.5×10^3

Contribution of parathymic lymph nodes to the amount of immunoglobulin isolated from thymus cells

Similar amounts of radiolabelled surface immunoglobulin were extracted from surface-labelled thymus and B-lymphocytes in agreement with earlier findings (Marchalonis *et al.*, 1972). The preparation of thymus-derived lymphocytes contained less than 0.2% B-cells as assessed by labelling experiments carried out with ^{125}I-labelled anti-θ serum to detect T-cells or anti-immunoglobulin under conditions that label B-cells selectively (D. Osmond, unpublished work).

The immunoglobulin obtained from our preparations of thymus cells is unlikely to be derived from cells of the parathymic lymph nodes, which could contribute to a maximum of 5–10% of the thymus cells present (E. Vitetta, personal communication). To test this possibility, further CBA mice were injected with india ink to make the parathymic lymph nodes visible, and 16h later their thymuses removed under magnification ×7.

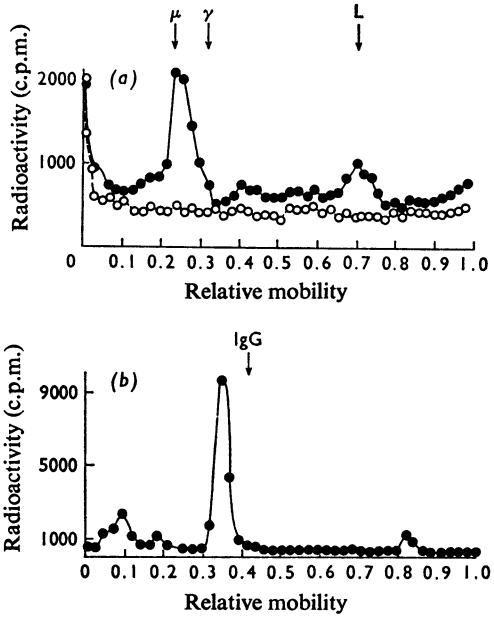

Fig. 1. *Polyacrylamide-gel electrophoresis in sodium dodecyl sulphate-containing buffer of ^{125}I-labelled polypeptide chains and intact molecules of surface immunoglobulin from CBA thymus lymphocytes free of parathymic lymph-node cells*

Specifically precipitated immunoglobulin was dissolved in sodium dodecyl sulphate in the presence or absence of 2-mercaptoethanol. Reduced samples were alkylated using iodoacetamide. (*a*) Reduced and alkylated surface immunoglobulin. ●, Rabbit antiserum to mouse IgG immunoglobulin plus mouse IgG; ○, fowl IgG plus rabbit antiserum to fowl IgG in 10% polyacrylamide, 0.25% bisacrylamide. (*b*) Unreduced surface immunoglobulin, in 5% polyacrylamide, 0.125% bisacrylamide. In Fig. 1(*a*), μ, γ and L refer to positions of standard immunoglobulin chains. In Fig. 1(*b*), IgG refers to the position at which mouse IgG migrates. Mobilities were expressed relative to Bromophenol Blue marker.

Cells (5×10^7) from each cell population, thymus lymphocytes contaminated with lymph-node lymphocytes and thymus lymphocytes free of lymph-node lymphocytes were surface-labelled with 1mCi of ^{125}I and extracted with 2.5ml of urea–acetic acid. After dialysis and centrifugation, cell-surface proteins were co-precipitated in 200μl fractions (4×10^6 cell equivalents). As shown in Table 2, co-precipitation of thymus lymphocytes containing lymph-node lymphocytes and thymus lymphocytes free of contaminating lymph-node lymphocytes revealed similar amounts of labelled immunoglobulin. The precipitates were dissolved in gel sample buffer as described in the Materials and Methods section and some were reduced and alkylated. Two major peaks of radioactivity were found in reduced, alkylated samples of specific precipitates of thymus lymphocytes free of contaminating lymph-node lymphocytes when the dissolved precipitates were resolved by disc-gel electrophoresis (Fig. 1*a*). Approx. 36000c.p.m. was added to the gel; approximately 33000c.p.m. penetrated into the gel. Over 85% of the specific counts in the gel were present in the two peaks, one of which migrated in the gel to the same extent as mouse immunoglobulin μ-chains, and the other which possessed the same R_F value as standard immunoglobulin light chains. When non-reduced specific precipitates were resolved by polyacrylamide-gel electrophoresis, three peaks were observed (Fig. 1*b*), representing 15% (R_F 0.1), 78% (R_F 0.35) and 7% of the specifically precipitated radioactivity. The major peak of radioactivity had an R_F value slightly lower than that of a mouse IgG standard (R_F 0.42). The results are consistent with those reported previously (Marchalonis *et al.*, 1972) that immunoglobulin molecules isolated from thymus lymphocytes had mol.wt. approx. 180000 and possessed μ-type heavy chains and light chains.

Isolation of surface immunoglobulin by detergent extraction

Two recent studies on the isolation of surface immunoglobulin from T- and B-lymphocytes by using enzyme-catalysed surface radioiodination have been unsuccessful in demonstrating surface immunoglobulin on T-cells (Vitetta *et al.*, 1972; Grey *et al.*, 1972). We believe that the discrepancy between our results and those reports were due to methodological differences. Most notably, Vitetta *et al.* (1972) and Grey *et al.* (1972) lysed surface-labelled lymphocytes with the non-ionic detergent Nonidet P-40. It was

thus thought possible that T-cell immunoglobulin was not extracted with this detergent. To test this possibility, 1×10^8 thymus or *nu/nu* spleen lymphocytes were radioactively labelled and extracted with 2ml of 0.5% Nonidet P-40 for 10min at 24°C (Baur *et al.*, 1971; Vitetta *et al.*, 1972). Approx. 51% of thymus lymphocyte [^{125}I]iodide-labelled cell-surface proteins were extracted by this procedure, whereas 72% of B-cell surface proteins were soluble. After dialysis against Tris–NaCl buffer, pH8.0, 10% of the detergent-soluble ^{125}I radioactivity associated with the surfaces of thymus cells or *nu/nu* spleen cells was retained. When cell-surface proteins were specifically precipitated with mouse IgG and rabbit antiserum to mouse IgG approx. 3.2% of the non-diffusible surface proteins obtained from *nu/nu* spleen cells were precipitated (Table 3). In contrast with the results obtained in the first section, no immunoglobulin was obtained from the radioiodinated thymus-cell-surface proteins extracted with Nonidet P-40.

Fractionation of T- and B-cell detergent-soluble cell-surface proteins

The results in the above section suggested that thymus- and B-lymphocyte membrane proteins differ in the degree to which they can be dissociated by Nonidet P-40. However, in several experiments the total amount of ^{125}I-labelled surface proteins released from T-lymphocytes by Nonidet P-40 was quite variable. Consequently, to characterize further the nature of surface proteins soluble in Nonidet P-40 or urea–acetic acid, samples of radioiodinated surface proteins obtained from thymus and *nu/nu* lymphocytes by both extraction procedures were reduced, alkylated and resolved in sodium dodecyl sulphate–polyacrylamide gels (Fig. 2). Different patterns were obtained for T- and B-lymphocytes extracted with Nonidet P-40. Thus lysates of surface-iodinated B cells were resolved into much more complex patterns with at least 13 distinct peaks. In contrast, resolution of Nonidet P-40 lysates of radioactively labelled T-cells yielded only five distinct major peaks.

Solubility of θ-antigen in Nonidet P-40 and in urea–acetic acid

We have determined whether the θ-alloantigen present on thymus cells (Raff, 1971) could be isolated by detergent extraction. For this 5×10^7 radioiodinated thymus lymphocytes or *nu/nu* spleen cells were extracted with urea–acetic acid or Nonidet P-40. In addition, 5×10^7 surface-labelled WEHI 105 thymoma cells (Harris *et al.*, 1973) were extracted with urea–acetic acid. Urea–acetic acid-dialysed lysates were first co-precipitated with mouse IgG and

Table 3. *Precipitation of immunoglobulin from detergent lysates of surface-labelled T- and B-lymphocytes*

Cells (1×10^8) were surface-labelled and lysed by suspension in 0.5% Nonidet P-40 for 10min at 24°C. After dialysis and centrifugation, 100μl of the supernatant was precipitated with mouse IgG+rabbit antiserum to mouse immunoglobulin or rabbit antiserum to mouse immunoglobulin+goat antiserum to rabbit IgG. Results represent the S.E.M. of four to six replicate samples, after five washes.

Cells	Radioactivity added (c.p.m.)	Radioactivity precipitated (c.p.m.)				Precipitation (%)	
		Direct		Indirect		Direct	Indirect
		Mouse IgG+rabbit antiserum in mouse immunoglobulin	Fowl IgG+rabbit antiserum to fowl IgG	Goat antiserum to rabbit IgG+rabbit antiserum to mouse immunoglobulin	Goat antiserum to rabbit IgG+normal rabbit serum		
nu/nu spleen	5.8×10^5	$2.1 \times 10^4 \pm 0.21 \times 10^4$	$2.5 \times 10^3 \pm 0.3 \times 10^3$	$3.6 \times 10^4 \pm 0.16 \times 10^4$	$1.4 \times 10^4 \pm 0.42 \times 10^4$	3.2	3.8
CBA thymus	4.1×10^5	$1.3 \times 10^3 \pm 0.18 \times 10^3$	$9.1 \times 10^2 \pm 0.6 \times 10^3$	$1.2 \times 10^3 \pm 0.14 \times 10^3$	$7.9 \times 10^2 \pm 2.4 \times 10^2$	0.1	0.1

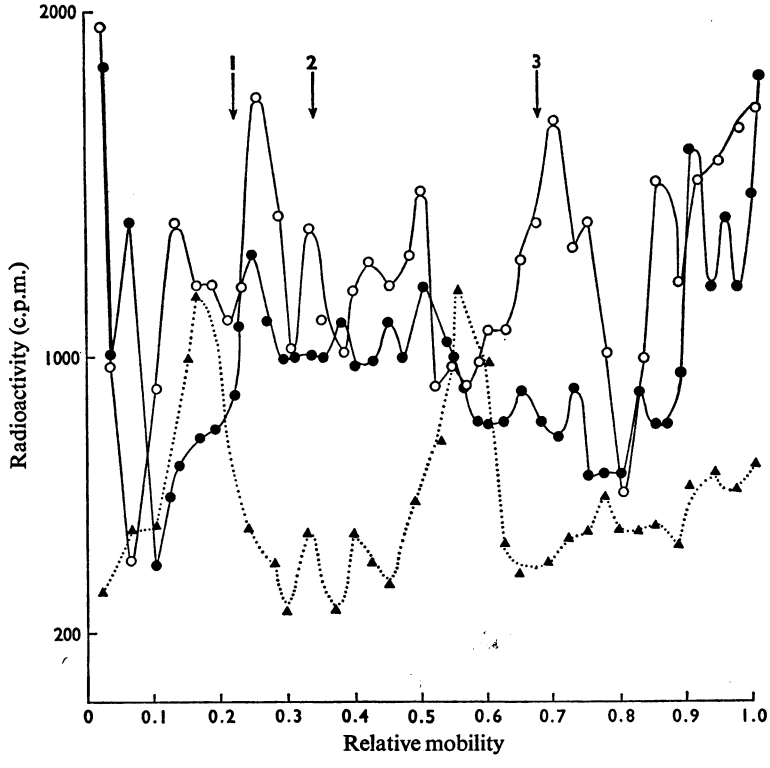

Fig. 2. *Comparison by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate buffer of ^{125}I-labelled membrane proteins extracted from CBA thymus lymphocytes or nu/nu spleen cells by urea–acetic acid or Nonidet P-40*

Samples (50μl) were reduced and alkylated and resolved on gel containing 10% polyacrylamide, 0.25% bisacrylamide. ○, B-cell surface proteins extracted with Nonidet P-40; ▲, thymus cell-surface proteins extracted with Nonidet P-40; ●, thymus cell-surface proteins extracted with urea–acetic acid. (1), The position of immunoglobulin μ chains, mol.wt. 70000; (2), γ chains, mol.wt. 50000; (3), light chains, mol.wt. 25000. Mobilities were expressed relative to Bromophenol Blue marker.

rabbit antiserum to mouse immunoglobulin to remove immunoglobulin. The supernatants of the first co-precipitation were pooled and 100μl samples were precipitated with mouse antiserum to θ of strain C_3H made in strain AKR and rabbit antiserum to mouse immunoglobulin and as controls normal serum of AKR strain mice or mouse IgG plus rabbit antiserum to this protein. As shown in Table 4, after four washes approx. 3% of the radioactivity was specifically precipitated by anti-θ antiserum+rabbit antiserum to mouse immunoglobulin. When urea–acetic acid extracts of *nu/nu* spleen cells were tested no specific precipitation of B-cell surface proteins with anti-θ serum was obtained from similarly treated preparations. Similar amounts of θ-antigen were precipitated from thymus cells lysed with Nonidet-P 40. In this case immunoglobulin was not removed before precipitation with anti-θ serum and rabbit antiserum to mouse immunoglobulin. No immunoglobulin was detected in this preparation. This was indicated by the fact that there was no difference between the radioactivity precipitated with normal serum of AKR strain plus rabbit antiserum to mouse immunoglobulin and that with the specificity control, fowl IgG plus rabbit antiserum to fowl IgG. In further experiments, Nonidet P-40 extracts of radioactively labelled CBA thymus cells were precipitated with AKR anti-$C_3H\theta$ antisera which had been absorbed previously with AKR or CBA brain homogenates. Antiserum which had been absorbed with AKR brain bound 15–20% less labelled surface proteins (5682c.p.m.) than non-absorbed antiserum (7481c.p.m.), whereas the amount of ^{125}I-labelled surface proteins bound by CBA-brain absorbed antiserum was decreased by more than 90% (688c.p.m.). Electrophoresis in sodium dodecylsulphate–polyacrylamide gels of specific precipitates obtained from thymus cells or thymoma

Table 4. *Isolation of θ-alloantigen by urea–acetic acid or detergent extraction*

Cells (5×10^7) were surface-labelled and lysed with urea–acetic acid or Nonidet P-40. Cell-surface proteins extracted with urea–acetic acid were co-precipitated first with mouse IgG+rabbit antiserum to mouse immunoglobulin. Then 200 μl portions of immunoglobulin-depleted urea–acetic acid-extracted surface proteins or non-immunoglobulin depleted Nonidet P-40 extracted surface proteins were precipitated with antiserum to $\theta(C_3H$ strain) made in AKR-strain mice or normal AKR serum plus rabbit antiserum to mouse immunoglobulin. Results represent radioactivity precipitated after four washes.

Cells	Treatment	Antiserum to $\theta(C_3H)$ made in AKR mice+rabbit antiserum to mouse immunoglobulin	Normal serum of AKR-strain mice+rabbit antiserum to mouse immunoglobulin	Fowl IgG+rabbit antiserum to fowl IgG	^{125}I-labelled macromolecular proteins specifically precipitated with anti-θ serum (%)
CBA thymus	Urea–acetic acid, immunoglobulin removed	3641	300	189	3.0
Thymoma WEHI 105	Urea–acetic acid, immunoglobulin removed	3270	1252	n.d.	1.5
nu/nu spleen	Urea–acetic acid, immunoglobulin removed	600	480	422	0
CBA thymus	Nonidet P-40, Immunoglobulin not removed	4892	248	330	2.6
nu/nu spleen	Nonidet P-40, immunoglobulin not removed	8400	9462	620	0

Fig. 3. *Resolution by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate-containing buffer of thymus cell-surface proteins specifically precipitated by AKR anti-$C_3H\theta$ antiserum and rabbit antiserum to mouse immunoglobulin*

(*a*) Cell-surface proteins extracted by urea–acetic acid; ●, CBA-thymus cells; ○, WEHI 105-thymoma cells. (*b*) Cell-surface proteins from CBA-thymus cells extracted with Nonidet P-40. μ, γ and L represent light-chain standards. Gels contained 10% polyacrylamide, 0.25% bisacrylamide. Mobilities were expressed relative to Bromophenol Blue marker.

cells showed in all cases a single component with mol.wt. approx. 60000 (Fig. 3), consistent with previous observations (Atwell *et al.*, 1973). The results indicate that whereas T-cell surface immunoglobulin is not solubilized by detergent extraction, θ-alloantigen is readily solubilized.

Discussion

The aims of this study were twofold: first, to obtain information on the molecular properties of two proteins (immunoglobulin and θ-alloantigen) known to be associated with the surfaces of certain lymphocytes, and second, to assess the effects of different solvents on the extraction of these proteins from the surfaces of either T- or B-lymphocytes. Immunoglobulin composed of light chains (mol.wt. 22000) and μ-like heavy chains (mol.wt. 70000) characterized by an intact mol.wt. of approx.

200000 was isolated from both T- and B-lymphocytes, after radioiodination of their surface proteins by catalysis with lactoperoxidase. A difference was observed between these two lymphocyte types, because treatment of B-cells with both acid–urea and the non-ionic detergent Nonidet P-40 extracted [^{125}I]iodide radioactivity specifically precipitated by antisera to immunoglobulin, whereas only the acid–urea treatment allowed successful detection of immunoglobulin from T-cells. The θ-alloantigen was extracted only from T-cells, and both acid–urea and Nonidet P-40 were equally effective in its isolation. This surface marker was obtained from both normal thymus lymphocytes and from the thymoma line WEHI 105. It existed predominantly as a component which exhibited a mobility on polyacrylamide-gel electrophoresis in sodium dodecyl sulphate-containing buffer consistent with a mol.wt. of 60000. A smaller component with an estimated mol.wt. approx. 20000 was occasionally observed.

A controversy has existed as to the presence of surface immunoglobulin on T-cells (Crone *et al.*, 1972), and certain workers have concluded from studies of radioiodinated T-cells extracted with Nonidet P-40 that T-cells either lack surface immunoglobulin or possess so little that it is undetectable (Vitetta *et al.*, 1972; Grey *et al.*, 1972). The present data, in conjunction with a variety of other approaches (reviewed by Marchalonis, 1974; Warner, 1974) including methodology similar to that described here (Moroz & Hahn, 1973), render that conclusion untenable. Our results establish that certain proteins of T- and B-cell membranes are differentially susceptible to dissociation with Nonidet P-40. This conclusion is consistent with a number of studies which show that non-ionic (Siekevitz, 1972; Keith & Mehlhorn, 1972; Cotman *et al.*, 1971) and ionic (Allan & Crumpton, 1971) detergents selectively isolate certain membrane-associated proteins, including enzymes. Moreover, electron-microscopic analysis has shown that profound structural and topographical differences occur between the surfaces of T- and B-lymphocytes (Mandel, 1972; Polliack *et al.*, 1973; Santer *et al.*, 1973). The study by Santer *et al.* (1973) demonstrated that thymus lymphocytes possess a glycoprotein cell coat nearly twice as thick as that of B-lymphocytes. This result is relevant to the present discussion because cell-coat components might compete with membrane components for [^{125}I]iodide, particularly where iodination conditions are sub-optimal. In accordance with this notion, Phillips & Morrison (1973) have shown that the glycoproteins of the erythrocyte surface can serve as a steric barrier which hinders the lactoperoxidase-catalysed radioiodination of surface proteins.

Work by Moroz & Hahn (1973) emphasizes another factor that might interfere with the isolation of surface proteins from surface-radioiodinated cells by extraction with non-ionic detergents. Solubilization of intact cells with Nonidet P-40 may release or activate cell-associated enzymes which then degrade proteins in the mixture. This possibility merits serious consideration because it is well known that the susceptibility of membrane proteins to proteolysis is enhanced in the presence of detergent (Steck & Fox, 1972). Similar problems could arise when urea–acetic acid is used as a solvent, if a sufficient number of enzyme molecules undergo renaturation after dialysis into aqueous medium. If proteolysis destroys T-cell immunoglobulin, the relative ease with which B-cell surface immunoglobulin can be isolated suggests that either T- and B-cell surface immunoglobulins may possess structural differences or T-cells possess more active proteases than do B-lymphocytes. The cytophilic properties of T-cell immunoglobulin for macrophages (Cone *et al.*, 1973) shows that functional and thus structural differences are found between surface immunoglobulins of T- and B-cells.

Other workers have recently isolated the θ-antigen by using techniques similar to those described here. Jones (1972) did not attempt to characterize the molecule, whereas Vitetta *et al.* (1973) reported that the θ-alloantigen had a mol. wt. of about 35000 and claimed that a glycolipid was crucial to antigenic integrity. A possible explanation for the discrepancy between their results and ours is that they used an antiserum prepared in congenic strains. Vitetta *et al.* (1973) suggest that an antiserum prepared in non-congenic strains might possess auto-immune antibodies which recognize thymus-specific antigens, unrelated to θ. However, this possibility appears to be ruled out by the observation that absorption of the antiserum used in our studies with CBA-strain brain abolished the activity of the antiserum whereas absorption with AKR-strain brain was without effect. It may be that antisera prepared in congenic and non-congenic mice recognize different antigenic moieties of the molecule and this, coupled with variations in extraction procedures, might lead to divergent results.

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