Immunology 1976 **31** 827

Mouse T-cell tumour immunoglobulin

I. ANTIGENIC PROPERTIES AND EFFECTS ON T-CELL RESPONSES

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Received 15 *April* 1976; *accepted for publication* 6 *May* 1976

Summary. The mouse T-cell tumours E1-4 and WEHI-22 produce an immunoglobulin (TCT Ig) which suppresses the antibody response to T-dependent antigens *in vitro* while having no effect on responses to T-independent antigens. TCT Ig also augments IgG responses to both kinds of antigen. TCT Ig appears to be a new class of mouse Ig because its effects on *in vitro* antibody responses can be absorbed by antisera to mouse Kappa chains but not with antisera to any of the known classes of mouse Ig. Furthermore, TCT Ig activity cannot be duplicated by free light chains, Fab_2 fragments, several mouse serum Ig preparations or Ig made from B cells.

TCT Ig affects only T-cell function in antibody responses. It has no effect on the ability of the different subclasses of T cells to respond to mitogens or to alloantigens. Furthermore, it does not block T–T-cell co-operation suggesting that the molecular basis for T–T co-operation differs from that of T–B co-operation.

INTRODUCTION

Thymus-derived (T) lymphocytes perform many different functions in the immune response. Among them are the helper effect in antibody responses to thymus-dependent antigens (Mitchison, 1971), stimulation of IgG production (Hay & Torrigiani, 1973), proliferative responses to allogeneic cells (Cerottini & Brunner, 1974) and the production of cytotoxic cells capable of killing allogeneic cells bearing the stimulating antigens (Wagner, Rollinghoff & Nossal, 1973). T cells can be divided into three separate subclasses by the Ly series of antigens expressed on their cell surface (Cantor & Boyse, 1975a). Cells bearing only the Ly1 antigen ($Ly1^+$) provide helper function (Kisielow, Hurst, Shiku, Beverley, Hoffmann, Boyse & Oettgen, 1975; Feldmann, Beverly, Dunkley & Kontiainen, 1975). Cells bearing the Ly2 and Ly3 antigens only ($Ly23^+$) produce the cytotoxic lymphocytes. A third population of lymphocytes bearing all three antigens ($Ly123^+$) exists and may represent immature precursor T cells destined to become either $Ly1^+$ or $Ly23^+$ cells. $Ly123^+$ cells proliferate when stimulated by cells bearing different major histocompatibility locus (MHL) antigens but have as yet no clearly defined function. $Ly1^+$ and $Ly23^+$ cells have a complex inter-relationship during the generation of cytotoxic cells (Cantor & Boyse, 1975b). Although $Ly1^+$ cells do not themselves become cytotoxic cells, they proliferate when stimulated by MHL differences and they augment the number of $Ly23^+$ killer cells produced. This sort of T–T-cell co-operation in

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which lymphocytes of one class assist the ability of cells of a different class to develop a function is reminiscent of co-operation between T and B cells in antibody responses.

T cell function during *in vitro* antibody responses can be influenced by an Ig which is produced by the T-cell tumours E1–4 and WEHI–22 (Feldmann, Boylston & Hogg, 1975; Stocker, Marchalonis & Harris, 1974). TCT Ig inhibits IgM responses to thymus-dependent antigens and augments IgG responses to both T-dependent and T-independent antigens. The mechanism by which TCT Ig causes these effects is unknown, but it may mimic the effects of a natural T-cell Ig which has been identified *in vivo* and *in vitro* (Feldmann, 1972; Taniguchi & Tada, 1974). Alternatively TCT Ig may affect T-cell function in some non-specific way such as inhibiting proliferation of T cells or by interfering with an unsuspected T–T interaction required for helper function (Feldmann, Erb, Kontiainen & Dunkley, 1975).

The experiments described in this paper are designed to answer two questions (1) What are the immunochemical properties of the active moiety in TCT Ig preparations? (2) What are the effects of TCT Ig on T-cell functions other than the helper effect?

The effects of TCT Ig on *in vitro* antibody responses provide a useful bioassay for immunochemical studies. Active preparations can be absorbed with insolubilized antisera to known proteins and the residual TCT Ig activity measured. T-cell function has been assessed by examining the ability of T cells to respond to mitogens or to allogeneic lymphocytes in the presence of TCT Ig. Co-operation between $Ly1^+$ and $Ly23^+$ cells has been measured by determining the number of cytotoxic cells produced by allogeneic stimulation and their capacity to kill appropriate target cells after stimulation in the presence of TCT Ig. Our results show that TCT Ig is a mouse Ig which appears to be immunochemically distinct from the known mouse Ig classes and that its effects are confined to T-cell functions in antibody responses.

MATERIALS AND METHODS

Animals

CBA/H, BALB/c, C57/Bl/6 and CBF_1 (C57 × BALB/c) mice were bred and maintained at St. Mary's Hospital Medical School. All animals were 16–28 weeks old when used.

T-cell lines and culture conditions

The T-cell tumours E1–4 and WEHI–22 have been described previously (Boylston, 1973; Harris, Bankhurst, Mason & Warner, 1973). They were maintained in tissue culture as described (Boylston & Mowbray, 1974).

Preparation of immunoglobulin from culture medium

Cultures were harvested when they had reached maximum cell density and the viability, measured by Trypan blue dye exclusion, had fallen to 85–90 per cent. Cells were removed by centrifuging at 1500 *g* for 20 min. The culture fluid was made 50 per cent saturated with ammonium sulphate (AS) by adding 313 g/l. A protease inhibitor, phenyl methyl sulphonyl fluoride (PMSF), was then added to a concentration of 50 μg/ml and the precipitate was removed by centrifuging at 7000 *g* for 30 min. The precipitate was dissolved and dialysed against 0·15 M NaCl 0·05 M Tris–HCl, pH 8·0 (NST) containing 50 μg/ml PMSF. The dialysed precipitate was centrifuged at 37,000 *g* for 30 min. Mouse Ig was purified from this material by affinity chromatography on columns of Sepharose 4-B insolubilized anti-mouse kappa chain antibody. The columns containing bound Ig were washed with three cycles of ten column volumes of 1 M NaCl, 0·05 M barbitone acetate, pH 8·9 followed by 1 M NaCl 0·05 M acetate, pH 5·0. The bound Ig was eluted by washing the column with five column volumes of 1 M propionic acid followed by three column volumes of 7 M guanidine, pH 2·0. The released protein was dialysed against NST and concentrated by vacuum dialysis to a protein concentration of 50 μg/ml. Yields of 30–50 μg protein per litre of culture fluid processed were obtained.

A control preparation was made from the culture fluids of normal human fibroblasts. This culture medium was processed by exactly the same method as the T-cell tumour culture fluids. However, after elution from the anti-kappa column, the protein concentration was unmeasurably low and therefore the volume was reduced to produce the same relative concentration as the TCT Ig preparations. A final volume of 1 ml for each litre of culture medium processed was used.

Preparation of serum proteins

The following mouse myeloma proteins have been used in this study. References given are to the purification methods employed. MOPC 104 (IgM) (Young, Jocius & Leon, 1971); 5563 (IgA2) (Potter, 1967); RPC (IgG2A) (Potter 1967); MPC 25 (IgG1) (Dissanayake & Hay, 1975); RPC5 urinary Bence–Jones protein (Kappa chain) (Potter, 1967); MPC 46B urinary Bence Jones protein (kappa chain) (Potter, 1967). MPC41 Bence–Jones protein was purchased from Bionetics Laboratories, Rockville, Maryland, U.S.A. The Fab_2 fragments of 5563 and MPC25 were produced by pepsin digestion as described (Potter, 1967).

Mouse spleen cell IgM was prepared by teasing 100 BALB/c spleens into cold Hanks's balanced salt solution (HBBS). The cells were washed three times with HBBS, and lysed in 0·15 M NaCl, 0·02 M PO_4, pH 7·2, containing 0·5 per cent NP_{40} with 50 μg/ml PMSF. The lysate was centrifuged at 10,000 *g* for 30 min and the supernatant filtered through a column of Sepharose 4-B insolubilized goat anti-mouse IgM antibody. Bound IgM was eluted with 7 M guanidine, pH 2·0, dialysed against NST, and concentrated by vacuum dialysis to a protein concentration of 70 μg/ml.

Bovine gamma globulin (BGG) was purchased from Sigma Chemical Company Ltd, Norbiton, London.

Antigens

Sheep red blood cells (SRBC) were obtained from Burroughs Wellcome Laboratories, Beckenham, Kent. DNP–POL was a gift from Dr Marc Feldmann, ICRF Tumour Immunology Laboratory, University College, London.

In vitro *primary antibody responses*

Antibody responses were generated by the modified Marbrook method (Feldmann, 1974). Antibody-forming cells (AFC) were detected by the Cunningham technique (Cunningham & Szenberg, 1968). IgM cells were developed directly. IgG-forming cells were developed by adding a rabbit anti-mouse IgG antiserum to the assay system. IgM responses were measured on day 4 and IgG responses on day 7. Results of these experiments are presented as the arithmetic mean AFC from quadruplicate cultures ± 1 standard deviation (S.D.). The data shown are AFC per slide; this figure can be converted to AFC per culture by multiplying by 20. TCT Ig or control preparations were added to cultures at the time of initiation.

Antisera

Anti-mouse kappa chain (RaK) antibody was produced by immunizing rabbits with several injections of 100 μg of a mixture of all three kappa chains listed above in Freund's complete adjuvant (FCA) obtained from Difco Laboratories, Detroit, Michigan. Pooled sera from several animals were absorbed with Sepharose-2B coupled BGG before further processing. Anti-mouse Fab_2 ($RaFab_2$) antibody was produced by immunizing a rabbit with 200 μg mixed Fab_2 fragments of 5563 and MPC25 in FCA. Both $RaFab_2$ and RaK react with free mouse kappa chain, Fab_2 fragments and intact Ig.

Goat anti-mouse IgM (GaM) was produced by immunizing a goat with purified MOPC 104 protein in FCA. Rabbit anti-mouse IgM (RaM) was prepared by immunizing rabbits with Sephadex G-200 void volume fraction of normal mouse serum in FCA. Sera from four animals were pooled before further processing. GaM and RaM were absorbed with Sepharose-4B insolubilized mouse IgG myeloma proteins, MPC25 and RPC5, and BGG before purifying the anti-IgM antibody.

Goat anti-mouse IgG1 (Ga1), IgG2A (Ga2a) and IgG2B (Ga2b) were gifts from Dr Richard Asofsky, Laboratory of Microbial Immunity NIAID, National Institutes of Health, Bethesda, Maryland, U.S.A. Rabbit anti-mouse IgG3 (Ra3) antisera were purchased from Bionetics Laboratories. Goat anti-mouse IgA (GaA) antiserum was purchased from Flow Laboratories Ltd, Irving, Scotland.

Goat anti-rabbit gamma globulin (GaRGG) was purchased from Calbiochem Ltd, Hereford. Rabbit anti-bovine serum (RaBS) was a gift from Dr J. F. Mowbray, St. Mary's Hospital Medical School, London.

RaK and $RaFab_2$ antibodies were purified by fractionating the antisera on Sepharose 4-B-coupled MPC25 protein. Bound antibody was eluted with 1 M PA and dialysed against NST. GaM and RaM were purified by chromatography on Sepharose 4-B-insolubilized MOPC 104 (IgM) protein. Bound antibody was eluted with PA and dialysed against NST.

Purified RaK, $RaFab_2$, GaM and RaM were coupled to Sepharose-4B as described (Givol, Weinstein, Gorecki & Witchek, 1970) at a protein concentration of 1 mg/ml bed volume. Gamma-

globulin preparations of the other antisera were prepared by two cycles of 50 per cent saturated AS precipitation. The precipitate was dialysed against NST and coupled to Sepharose 4-B as described.

Antibody to E1–4 ($H2^b$) transplantation antigens was raised by immunizing female BALB/c mice with weekly injections of 3×10^7 cultured E1–4 cells. One week after the fourth injection the animals were bled and the serum shown to have an anti-C57 spleen cell titre of 1:320. Antibody to WEHI-22 ($H2^b$) transplantation antigens was raised by immunizing C57 female mice with cultured P815Y mastocytoma cells, an $H2^d$ bearing cell line. The serum was harvested after six weekly injections and shown to have an anti-BALB/c spleen cell titre of 1:160. Fifty per cent AS precipitated fractions of both sera were prepared and coupled to Sepharose-4B.

Immunochemical analysis

TCT Ig was incubated with 0·1 ml bed volume of Sepharose 4-B-coupled antibody at 4° for 48 h on a rotating mixer. The absorbent was removed by centrifuging at 1500 *g* for 10 min. The supernatant was removed and sterilized by filtration through a 0·22 μm pore size Millipore filter (Millipore Corporation U.K. Ltd, London). When appropriate, the absorbent was preincubated with 100 μg of purified MPC25 protein at 37° for 24 h before using it to absorb TCT Ig.

T-cell responses in vitro

BALB/c mesenteric lymph nodes (MLN) were teased into HBSS buffered with 0·01 M Hepes buffer containing 10 per cent foetal bovine serum (FBS). Fragments were allowed to settle at room temperature for 10 min and the suspensions were washed twice in RPMI 1640 buffered with 0·01 M Hepes buffer supplemented with 50 mg/l cloxacillin, 40 mg/l gentamycin, 2·0 g/l $NaHCO_3$ and 7½ per cent fresh normal human serum (1640-NHS) which had been heated at 56° for 30 min. Cells were resuspended in 1640-NHS at a concentration of 3×10^6 viable cells/ml (determined by trypan blue dye exclusion) and cultured in 1-ml volumes in 9 × 44 mm sealed plastic tubes (Raven Scientific Ltd, Haverhill, Suffolk). Phytohaemagglutinin (PHA) at a final concentration of 1·25 μg/ml, Concanavalin A (Con A) at a final concentration of 2·0 μg, or diluent were added before culture. After 48 hours in culture 1 μCi [^{3}H]-thymidine (Radiochemical Centre, Amersham, Bucks) was added and culture was continued for 1 h. Labelled cells were harvested by filtration on to glass fibre filters, washed with methanol and trichloracetic acid, and counted in a scintillation counter.

One-way mixed lymphocyte cultures (MLC) were initiated by mixing $1{\cdot}5 \times 10^6$ BALB/c MLN cells with $1{\cdot}5 \times 10^6$ CBF_1 MLN cells in 1640-NHS in sealed vials as above. After 72 h in culture 1 μCi-[^{3}H]TdR was added and the cultures were harvested and counted as above. The results of these experiments are expressed as the arithmetic mean c.p.m.[^{3}H]TdR incorporated ± s.d.

Cytotoxic capacity of MLC cells

BALB/c ($H2^d$) and anti-C57 ($H2^b$) cytotoxic lymphocytes were generated in one way MLC as above.

After 6 days in culture 20–30 1-ml cultures were pooled, washed once in 1640-NHS and resuspended in 1640 5% FBS. The number of viable cells remaining in each pool was determined by counting the number of trypan blue dye-excluding cells in a haemocytometer. The ability of different numbers of these cells to kill ^{51}Cr-labelled E1–4 ($H2^b$) target cells was measured by the method of Brunner, Mauel, Rudolf & Chapuis (1970). Maximum target cell lysis was determined by incubating an aliquot of ^{51}Cr-labelled E1–4 cells in PBS 1·0 per cent Triton × 100 instead of stimulated lymphocytes. The results of these experiments are reported as the arithmetic mean c.p.m ^{51}Cr released from triplicate cultures ± s.d.

RESULTS

Effect of T-cell tumour immunoglobulin on *in vitro* immune responses

The effect of TCT Ig on the *in vitro* immune response to a thymus-dependent antigen, SRBC, and to a thymus-independent antigen, DNP–POL, is shown in Table 1. Both E1–4 Ig and WEHI–22 Ig suppress the IgM response to SRBC on day 4 but do not affect the response to DNP–POL. The IgG response on day 7 to both antigens is augmented. A dose–response curve for IgM anti-SRBC suppression by TCT Ig is shown in Fig. 1. Near maximum suppression was produced by a concentration of 1 μg/ml TCT Ig. Therefore this concentration was chosen for all further studies. Since both IgM suppression and IgG stimulation can be demonstrated using SRBC as the antigen it was chosen for use in further experiments.

Table 1. Effects of E1–4 Ig and WEHI–22 Ig on the *in vitro* immune response to SRBC

Immunoglobulin added (1 μg/ml)	IgM AFC (day 4)		IgG AFC (day 7)	
	SRBC	DNP–POL	SRBC	DNP–NOL
None	101±12	78·5±5	2·5±0·5	2·5±5
WEHI-22	44·5±6	73±10	42±6	39±4
E1-4	56·5±7	84±2·5	24·5±10	29±9

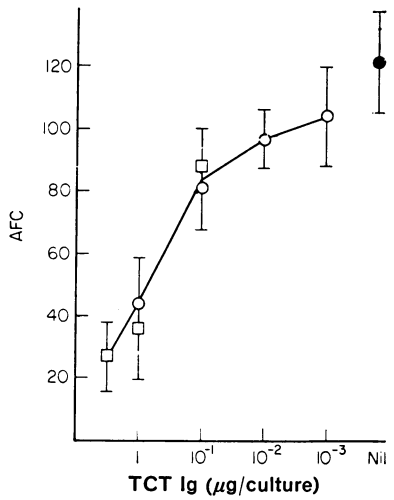

Figure 1. Dose–response curve for the suppression of IgM anti-SRBC AFC by TCT Ig. (□) EL-4; (○) WEHI-22; (●) control.

Table 2. Absorption of biologically active TCT Ig with insoluble antibodies to mouse Ig, rabbit Ig, or bovine serum

TCT Ig added (1·0 μg/ml)	Insoluble absorbent	IgM PFC (day 4)	IgG PFC (day 7)
None	—	137±11	2·0±1
WEHI–22	None	57±15	23·5±3
	RaK	119±15	4·3±1·5
	$RaFab_2$	113±8	1·8±0·9
	RaK+MPC 21 (IgG1)	85±9	23·3±2
	GaRGG	62±4	21·5±5
	RaBS	37·5±10	24±4
None	—	104±9	1·8±1
E1–4	None	47·5±5	34±3·5
	RaK	85±7·6	3±1
	RaK+MPC 21 (IgG1)	40·8±10	20·5±1·7
	GaRGG	55·7±7	23·3±5
	RaBS	57±7	17·3±3

Presence of mouse light chains in TCT Ig

These experiments were performed to show that the molecule responsible for the effects of TCT Ig on the *in vitro* antibody response contains mouse light chains. WEHI–22 Ig and E1–4 Ig were incubated with insoluble antisera to mouse kappa chains or to control proteins and the residual effects on antibody responses measured. The results are shown in Table 2 where it is seen that anti-kappa and anti-Fab_2 antibodies completely remove the ability of TCT Ig to suppress IgM responses or to augment IgG responses. Absorption of TCT Ig was specific because it could be blocked by preincubating the anti-kappa reagents with pure mouse IgG. Antibody to rabbit gamma globulin or bovine serum had no effect on TCT Ig functions.

Attempts were made to identify a heavy chain in TCT Ig by absorbing E1–4 Ig or WEHI–22 Ig with antisera to the known mouse Ig classes. The anti-M absorbent consisted of equal volumes of RaM and GaM described above; the anti-G absorbent was composed of equal volumes of insolubilized Ga1, Ga2a, Ga2b and Ra3. These reagents did not absorb either the IgM suppression or the IgG stimulating properties of TCT Ig, as shown in Table 3.

The effect of insolubilized antisera to the major histocompatibility locus of the cell lines producing TCT Ig was investigated. These antisera do not react with TCT Ig from the same or unrelated strains (Table 4).

Since anti-light chain but not anti-heavy chain antibodies react with TCT Ig we attempted to reproduce its biological properties with free mouse kappa

Table 3. Attempts to absorb the biological activity of TCT Ig with antisera to mouse Ig heavy chain classes

TCT Ig added (1·0 μg/ml)	Insoluble absorbent	IgM AFC (day 4)	IgG AFC (day 7)
None	—	103·7±8	1·3±1
WEHI-22	None	64·5±8	35±6
	Anti-IgM	53·6±6	25±8·7
	Anti-IgG	58·3±18	24·8±5
	Anti-IgA	63·3±5	22·5±8
E1-4	None	37·5±5	31±13
	Anti-IgM	43·8±9	26±6·6
	Anti-IgG	53·8±12	24·8±7
	Anti-IgA	45·5±5	28±6·5

Table 4. Failure to absorb TCT Ig activity with antisera to the major histocompatibility locus of the producer cell line

TCT Ig added (1·0 μg/ml)	Absorbent	IgM AFC (day 4)
WEHI-22	Anti-BALB/c	72±9
	Anti-C57	72±13
	Anti-K	127±20
E1-4	Anti-BALB/c	75±15
	Anti-C57	66±11
	Anti-K	113±17
None	—	128±16

Table 5. Effects of mouse Bence-Jones proteins, Fab_2 fragments, spleen IgM and human fibroblast supernatant on the primary *in vitro* immune response to SRBC

Protein added	IgM anti-SRBC plaques (day 4)	IgG anti-SRBC plaques (day 7)
None	103±9	7·8±3
WEHI-22 Ig	59±15	21·5±6
MOPC 46B	115±16	6·3±6
MPC 41	115±17	6±3
5563 Fab_2	97·5±6	4·3±3
MPC 25 Fab_2	98±8	2±2
Mouse spleen IgM	150±10	4·3±3
Human fibroblast supernatant	130±15	1·8±2

chains and Fab_2 fragments. We also investigated whether TCT Ig-like activity could be extracted from the culture supernatant of normal human fibroblasts, or whether similar effects could be produced by B-cell IgM. In Table 5 the results of these experiments are shown; none of these proteins has TCT Ig-like effects.

Effects of TCT on T-cell functions

Different T-cell subsets respond to different mitogenic stimuli. Con A stimulates $Ly1^+$ cells to proliferate while PHA stimulates $Ly123^+$ cells. Allogeneic lymphocytes induce proliferation in $Ly1^+$ and $Ly23^+$ and $Ly123^+$ subsets. We have investigated the effects of TCT Ig on these T-cell proliferative responses. In Table 6 the absence of any effect of TCT Ig on [^{3}H]TdR incorporation induced by these stimuli is shown.

Co-operation between $Ly1^+$ and $Ly23^+$ cells in the production of cytotoxic lymphocytes was studied by measuring the number of viable cytotoxic cells and their cytotoxic capacity following one way MLC. TCT Ig does not affect the number of viable cells produced after 6 days in culture (Table 7). The ability of these cells ($H2^d$ anti-$H2^b$) to kill ^{51}Cr-labelled E1-4 cells ($H2^b$) is shown in Table 8. Cells

Table 6. Effect of TCT Ig on the proliferation of mouse lymphocytes induced by Pha, Con A or MLC

Mitogenic stimulus	[^{3}H]TdR (c.p.m. incorporated)		
	WEHI-22 Ig	E1-4 Ig	Diluent
Pha	88,645±4654	87,811±12,965	79,784±12,005
Con A	86,932±9742	93,609±12,657	90,729±14,198
MLC	8328±1066	10,090±997	9111±1032

Table 7. Number of viable cells on day 6 following allogeneic stimulation of mouse lymphocytes in the presence of TCT Ig

TCT Ig added (1·0 μg/ml)	Viable cells ($\times 10^6$)
None	6·06±0·88
E1-4	5·8±0·22
WEHI-22	6·36±0·38

Table 8. Cytotoxic capacity of lymphocytes stimulated in one-way MLC in the presence of TCT Ig

	TCT Ig added (1·0 μg/ml)*		
Killer/target ratio	None	WEHI–22 Ig	E1–4 Ig
30/1	1108±72	1074±101	897±70
15/1	986±60	1101±131	1043±42
7·5/1	824±61	836±162	742±61
3.75/1	609±60	575±103	654±141
100% Lysis	2245±120		
Spontaneous release	160±43		

* Values presented are mean ^{51}Cr c.p.m. released from triplicate cultures ±s.d.

grown in the presence of WEHI–22 Ig or E1–4 Ig possessed the same cytotoxic capability as normal cells at several different killer/target ratios which is further evidence that TCT Ig does not influence T–T-cell cooperation.

DISCUSSION

These experiments show that the mouse tissue cultured T-cell tumours, E1–4 and WEHI–22, produce an Ig which has important effects on primary *in vitro* antibody responses. The active factor is a mouse Ig because it can be absorbed with insoluble antibodies that react with mouse kappa chain and this absorption can be blocked by preincubating the immunosorbent with pure mouse IgG. We have shown that the active factor is not rabbit anti-kappa antibody eluted from the preparative Sepharose anti-kappa column because it does not react with antibody to rabbit γ-globulin or to mouse IgG which is bound to RaK in the blocking experiment shown in Table 2. The active moiety is not a component of the foetal bovine serum used in the T-cell tumour cultures because it cannot be absorbed with antibody to bovine serum nor can TCT Ig activity be produced by processing culture medium containing the same batch of FBS from human fibroblasts in exactly the same way as T-cell culture supernatant.

Our attempts to characterize the heavy chain of the TCT Ig suggests that this Ig belongs to a previously undescribed class of Ig because antisera to the known mouse Ig heavy chain classes do not absorb the biological activity of TCT Ig. Two possible objections to this conclusion are that the reagents used are inadequate, or that the procedure used to prepare TCT Ig has denatured the class-specific antigenic determinants. The following observations make these objections unlikely.

Both anti-IgM reagents used in these experiments have also been used in our studies on B-cell surface immunoglobulin. Both reagents react with all of the B-cell surface IgM indicating that they recognize the antigenic determinants present on all μ chains. The anti-IgG1, 2a and 2b class-specific reagents have been characterized by Dr R. Asofsky (personal communication). The anti-IgA and anti-IgG3 reagents are commercially available. In addition, IgM and individual IgG subclasses prepared by guanidine elution from the bound antisera used in these experiments retain their ability to react with the appropriate antisera. Therefore, our reagents recognize the known classes and subclasses of mouse Ig and preparations of known Ig classes by the methods used to prepare TCT Ig, yield antigenically intact proteins. Final immunochemical characterization of TCT Ig must await production of an antiserum to it which will allow its exact antigenic relationship to other mouse Ig to be determined.

The active agent in TCT Ig does not react with antibody directed towards the histocompatibility antigens of the cells producing it.

Our chemical studies of TCT Ig show that it has a two chain structure and that it is composed of a typical light chain and a heavy chain with a mol. wt. of about 60,000 (Boylston & Mowbray, 1974).

Further evidence that TCT Ig may be a new class of Ig is derived from failure to reproduce its biological properties with representatives of the recognized mouse Ig proteins. Myeloma proteins or heterogeneous mixtures of serum Ig or Ig extracted from nude mouse spleen cells, have been shown to be ineffective (Feldmann *et al.*, 1975). We have extended the list of inactive proteins to include free light chains, Fab_2 fragments, and an IgM preparation extracted from normal mouse spleen cells.

Recently an Ig-binding protein released by stimulated T cells which can suppress *in vitro* antibody responses has been described (Fridman, Neauport-Santes, Guimezanes & Gisler, 1975). This molecule is unlikely to be responsible for TCT Ig activity because it also suppresses the response to T-independent antigens, whereas TCT Ig has no effect on such responses.

Our data show that TCT Ig only affects those cell functions directly concerned in antibody responses.

Since it does not depress [^{3}H]TdR incorporation during stimulation by MHL differences, PHA or Con A, it does not affect the ability of Ly1$^+$, Ly23$^+$ or Ly123$^+$ cells to proliferate (Hurst, Beverly, Kisielgow, Hoffman & Oettgen, 1975). The number and killing capacity of cells generated in MLC are also unaffected showing that TCT Ig does not block co-operation between Ly1$^+$ and Ly23$^+$ cells, nor does it affect the development of effector function by Ly23$^+$ T cells. B-cell responses are unaffected because the antibody response to T-independent antigens which stimulate B cells directly is unchanged. Therefore, TCT Ig acts only on those steps in cooperative antibody responses which lie between the ability of the T cell to respond to antigen and the ability of the B cell to produce antibody.

A T-cell Ig (IgT) which mediates antigen specific cooperation between T and B-cells *in vivo* and *in vitro* has been described (Feldmann, 1972; Taniguchi & Tada, 1974). This Ig is thought to be released by T cells and to bind to macrophages as an IgT–antigen complex. The macrophage-bound antigen IgT complex stimulates B cells to make IgM responses. In this model TCT Ig should act by inhibiting the IgM response to thymus-dependent antigens by competing for the macrophage receptor site required for antigen-specific co-operative IgT binding. Excess specific IgT can also have the same effect (Feldmann, 1974). One important difficulty in ascribing TCT Ig activity to competition between it and a natural antigen-specific T-cell Ig is that the natural T-cell Ig reacts with anti-IgM antisera, and TCT Ig cannot be absorbed with antisera to any of the known mouse heavy chain classes. One explanation for this discrepancy may be that T-cell Ig shares only a few antigenic determinants with IgM and is only recognized by some anti-IgM antisera. Similar cross-reactions between human Ig classes are well documented (Low, Liu & Putnam, 1976). This explanation seems likely since our anti-IgM reagents react with all the mu chains present on mouse B cells.

Further elucidation of the relationship between TCT Ig and possible immunoglobulin factors involved in cooperative immune responses will require production of an antiserum specific for IgT.

ACKNOWLEDGMENTS

This project was supported by grants from the Medical Research Council. The authors would like to thank Drs M. C. Berenbaum and J. F. Mowbray for the use of laboratory facilities.

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