# IMMUNOLOGICAL ACTIVITIES OF A LYMPHOCYTE MITOGEN ISOLATED FROM COENURUS FLUID OF *TAENIA MULTICEPS* (CESTODA)

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**Summary :**

The purification of a mitogen from *Taenia multiceps* coenurus fluid has been previously reported. In the present study, this activity, which was independent of endotoxin, stimulated the expression of lymphocyte IL-2 and Fc receptors, enhanced mitotic response to phytohaemagglutinin and concanavalin A and antagonised the previously described suppressive effects of the macrophage modifying fraction of coenurus fluid. The mitogen also increased peritoneal macrophage count and viability, Fc receptor expression and Fc receptor-mediated phagocytosis. The mitogenic activity could be destroyed by a combination of protease and amylase, but not by either enzyme alone. It is suggested that the mitogen forms part of a homeostatic mechanism for the preservation of a balanced host-parasite relationship.

**KEY WORDS :** *Taenia*, coenurus, immunoregulation, macrophage, T cell, Fc, C3b, histocompatibility antigen, phagocyte, enzyme, *Echinococcus*, hydatid.

***Résumé :*** LES EFFETS IMMUNOLOGIQUES D'UN MITOGÈNE LYMPHOCYTAIRE ISOLÉ DU FLUIDE DU COENURUS DE *TAENIA MULTICEPS* (CESTODA)

*Un mitogène de fluide de cénure de* Taenia multiceps *a été purifié antérieurement. Au cours de ce travail, il est montré que l'effet mitogène était indépendant de l'endotoxine, stimulait l'expression des récepteurs lymphocytaires pour l'IL2 et le fragment Fc des immunoglobulines, augmentait la réaction mitogène induite par la phytohaemagglutinine et la concanavaline-A et s'opposait aux effets suppressifs, déjà rapportés, de la fraction 24 de fluide de cénure. Le mitogène augmente aussi le nombre, la viabilité, l'expression des récepteurs Fc et la phagocytose via le Fc des macrophages péritonéaux. L'effet du mitogène est inhibé par l'action conjointe d'une protéase et de l'amylase, mais par aucune de ces enzymes prises isolément. Ceci suggère que le mitogène participe à un mécanisme d'homéostasie pour la conservation d'un rapport équilibré entre l'hôte et le parasite.*

***MOTS CLÉS :*** Taenia, *coenurus, immunorégulation, macrophage, cellule T, Fc, C3b, histocompatibilité, phagocyte, enzyme,* Echinococcus, *kyste hydatique.*

## INTRODUCTION

Immunoregulators in metacestode infection have been reviewed by Dixon & Jenkins (1995*a, b*). Several authors have suggested that mitosis enhancement by metacestodes subverts protective T-cell function by, for instance, the generation of T-suppressor activity (Riley *et al.*, 1987; Kizaki *et al.*, 1991, 1993*a, b, c*). A mitogenic factor has previously been isolated from *Taenia multiceps* coenurus fluid (TMCF) by ion exchange chromatography. This isolate stimulated a distinct L3T4$^+$ population of target cells, and accentuated macrophage-mediated lymphocyte responses to plant mitogens, while at the same time rendering lymphocyte mitosis more susceptible to macrophage-mediated suppression (Rakha *et al.*, 1991*a, b*). The TMCF mitogen, probably identical to a 40 kDa. component described by Judson *et al.* (1987), is chromatographically distinct from another immunoregulatory factor in the coenurus which modifies the accessory activity of macrophages (Rakha *et al.*, 1991*b*, 1996). The chemical nature and cellular effects of the mitogen are important for understanding its possible effect on T-cell- and macrophage-mediated protective immunity. This communication reports enzyme digestion experiments on the mitogen, its effects on expression and function of Ia determinants and IL-2, Fc and C3b receptors, and its ability to antagonise the suppressive effects of macrophage modifying factor on lymphocyte mitosis.

## MATERIALS AND METHODS

### *T. MULTICEPS* PRODUCTS

Coenuri of *T. multiceps* were obtained in the course of surgical treatment of naturally infected sheep. Coenurus fluid fraction 7, a T-cell mitogen, and 24, a lymphoproliferation inhibitor, were purified by FPLC as described by Rakha *et al.* (1991*b*).

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### Animals

Normal, grazing sheep were used as donors of the blood lymphocytes for the enzyme digestion assays (see below). All other experiments on leucocyte culture used mesenteric lymph-node cells from BALB/c mice kept under standard laboratory conditions. In some experiments these cells were modified by intraperitoneal injection of *T. multiceps* products prior to collection and purification.

### Enzyme digestion assay

The assay was conducted as described by Judson (1987) with the enzymes listed in Table I. Two ml of TMCF fraction 7 were mixed with 2.0 ml of enzyme dissolved in RPMI tissue culture medium (TCM, see below) and incubated under various conditions (Table I). When a combination of enzymes was used, the TMCF-fraction 7 sample was treated with the first enzyme under optimal conditions. The digest was frozen, thawed and treated by the second enzyme. After incubation, the resultant digest was frozen at – 20 °C for at least 24 h, thawed and then tested for mitogenic effect on sheep peripheral-blood lymphocyte cultures as described by Rakha *et al.* (1992). Control samples consisted of equal parts TMCF-fraction 7 and TCM and were incubated for 24 h before freezing.

### Endotoxin detection

*T. multiceps* protoscoleces are a potent source of lymphocyte mitogen (Judson *et al.*, 1984, 1987). To determine whether this mitogenic action was the result of protoscolex-borne endotoxin, protoscoleces were washed in endotoxin-free water (Sigma, UK) and homogenised in a sterile, siliconised tissue grinder. Endotoxin was detected by the limulus assay (Sigma) according to the maker's instructions (Elin *et al.*, 1976).

### Polymixin B treatment

Apart from the above enzyme digestion assays using sheep lymphocytes, the majority of experiments reported here involved the modification of leucocytes by *in vivo* injection of *T. multiceps* products. Since this procedure required murine experimental hosts, a preliminary test was made of the possible participation of endotoxin in the mitotic activation of murine lymphocytes by *T. multiceps* products. As the mitotic action of endotoxin lipopolysaccharide can be abolished by Polymixin B (Jacobs & Morrison, 1975), murine mesenteric lymph-node cells (see below) were incubated with (TMCF) fraction 7 with or without Polymixin B (Sigma) at 100 or 500 units per culture, then assayed for lymphoproliferation by $^{3}$H-thymidine incorporation as described by Dixon *et al.* (1982) and Rakha *et al.* (1991*a*).

### Peritoneal macrophages

Normal or parasite-activated peritoneal macrophages were collected from BALB/c mice (Rakha *et al.*, 1991*a*, *b*). The abundance and viability of cells was assessed by counting at least 500 cells in 0.2 % trypan blue in a haemocytometer. For *in vivo* activation of macrophages with *T. multiceps* products, mice were injected intraperitoneally twice at 7 days interval with TMCF (0.6 ml) or TMCF fraction 7 (0.2 ml). Control mice were injected with equivalent amounts of phosphate-buffered saline pH 7.2 (PBS). Cells were collected 3 days after the second injection, or at other time intervals indicated in Results. Macrophages used in Fc- and C3b-receptor assays and viability counts were incubated in tissue culture medium (TCM) consisting of RPMI-1640 (Northumbria) containing 20 mM HEPES, 2 mg/ml sodium bicarbonate (Sigma), 2 mM glutamine (Sigma), 50 µg/ml gentamycin (Sigma), 3 µg/ml amphotericin (Sigma), 5 % neonatal calf serum (Sigma), 100 mM sodium pyruvate (Sigma) and $10^{-5}$ M 2-Mercapto-ethanol (BDH, UK). Macrophages (1 or $2 \times 10^{6}$/ml) were cultured in 24-well microtitre plates (Northern Media, UK) at 37 °C in 5 % $CO_2$. Sterile glass cover slips were placed in the wells in advance. The number of cells which formed cytoplasmic processes in culture was also recorded. For rosetting assays, coverslips were removed after 2 h incubation, washed in RPMI-1640 at 37° and placed in fresh wells for the assay. For *in vitro* activation of Ia expression, macro-

| Enzyme | Type/Source | Concentration (Units/ml or µg/ml) | Temp. (°C) | Time (h) | pH |
|---|---|---|---|---|---|
| Trypsin | 1/bovine pancreas | 100 µg | 25 | 4 | 7.6 |
| Protease | *Streptococcus griseus* | 10 U | 37 | 2 | 7.5 |
| Neuraminidase | VI A/*Clostridium perfringens* | 5 U | 37 | 24 | 5.0 |
| Lipase | VIII, *Candida cylindracia* | 250 U | 37 | 2 | 7.5 |
| Amylase | II A, *Bacillus* spp. | 150 U | 20 | 4 | 6.9 |

Table I. — Enzymes used for digestion of TMCF-fraction 7, showing optimal concentrations, pH, time and temperature of incubation.

phages were incubated with 10 % by volume of TMCF or TMCF fraction 7 (Rakha *et al.* 1996).

### MESENTERIC LYMPH-NODE CELLS

Mice injected according to the above schedule were also used for collection of mesenteric lymph nodes, which were sieved to produce suspensions of lymph-node cells (Rakha *et al.*, 1991*b*).

### FC RECEPTOR (FCR) EXPRESSION BY MACROPHAGES

IgG and IgM fractions of a rabbit antiserum against sheep red cells (SRBC) were prepared by fractionation on an ACA 34 gel filtration column (Pharmacia). SRBC (5 %) were incubated with anti-SRBC IgG in PBS at 37 °C for 30 min, the dilution of the IgG being adjusted by previous experiment to produce SRBC which formed rosettes with 5 % of normal peritoneal macrophages. Antibody-coated SRBC were washed in PBS, suspended in veronal-buffered saline at 5 % and introduced in 250 µl volumes into wells of 24-well tissue culture plates. Each well contained a cover slip bearing a monolayer of approximately $10^5$ washed macrophages. Monolayers were incubated with the IgG-treated SRBC for 1 h at 37 °C, washed three times with sterile PBS, fixed in 2 % glutaraldehyde and stained with Wright-Giemsa. Rosettes, defined as macrophages carrying at least 3 SRBC, were counted in populations of at least 150 cells per coverslip, and at least 500 cells per macrophage sample.

### C3B RECEPTOR (C3BR) EXPRESSION BY MACROPHAGES

The IgM component of the anti-SRBC rabbit serum was incubated at a subagglutinating concentration with 5 % SRBC for 30 min at 37 °C. SRBC were washed three times and resuspended in 1.0 ml of veronal buffered saline containing 0.1 ml of fresh normal rat serum as a complement source, and 0.1 ml of suramin solution (2.0 mg $ml^{-1}$). After mixing and incubating for a further 10 min at 37 °C, C3b-coated SRBC were washed in PBS and resuspended in 5 ml Veronal-gelatin buffer then applied to macrophage monolayers.

### FCR AND C3BR-MEDIATED INGESTION ASSAY

Macrophage immunophagocytic function was assessed by using either Fc or C3b receptor-mediated ingestion activity. Washed SRBC were opsonized as above with either IgG (FcR mediated phagocytosis) or with IgM and fresh serum (C3bR-mediated phagocytosis). Ingestion controls consisting of SRBC alone or SRBC+IgM without complement were used to quantify non-specific ingestion. Macrophage monolayers were incubated with the SRBC preparations for 2 h at 37 °C. Positive macrophages, defined as those containing two or more ingested SRBC, were counted in populations of 500 per slide. Results were reported as % phagocytosing cells after subtraction of the appropriate scores for non-specific ingestion.

### FCR AND C3BR EXPRESSION BY LYMPHOCYTES

FcR and C3bR on lymphocytes were quantified using suspensions of lymph-node cells as described by Rakha *et al.* (1991*b*).

### IA EXPRESSION BY MACROPHAGES

The percentage of macrophages bearing the Ia determinant was measured by immunofluorescent staining using a biotinylated, anti-Ia monoclonal antibody, Serotec (Clone No., H116-32-R5) followed by flow cytometric analysis on a FACS 420 (Becton-Dickenson), as described by Rakha *et al.* (1996).

### IL-2 RECEPTOR EXPRESSION BY LYMPHOCYTES

The percentage of lymphocytes bearing IL-2 receptor was measured by immunofluorescence employing biotinylation and flow cytometry as above. The anti-IL2R monoclonal antibody was MRC OX 39 (Serotec).

### STATISTICS

The significance of differences between receptor expression in different cell populations was calculated from $\chi^2$ determinations based on two-by-two contingency tables. Peritoneal cell counts were compared by Student's *t* test. Other comparisons between groups were made by the Mann-Whitney U test, since this method does not assume normal distribution of the data.

## RESULTS

### ENDOTOXIN ASSAY OF *T. MULTICEPS* PROTOSCOLECES

To distinguish between parasite-derived mitogenic signals and possible interference from endotoxin, homogenates of protoscoleces were screened for endotoxin activity. No activity could be detected in any homogenate despite the ability of the asssay to detect endotoxin at concentrations below 1.0 pg/ml.

### MITOGENIC ACTION OF *T. MULTICEPS* FRACTION 7: EFFECT OF POLYMIXIN B

Triplicate cultures of BALB/c mesenteric lymph-node cells at 1.0, 1.5 or $2 \times 10^6$ cells/ml were incubated with 10 % by volume of TMCF fraction 7 with or without Polymixin B. No significant effect of Polymixin B was

evident from ranking the resultant cpm values (results not shown). These experiments therefore failed to detect the presence of endotoxin in *T. multiceps* preparations.

### Lymphocyte expression of IL-2 receptor: Enhancement by TMCF fraction 7

Figure 1 shows the results of flow cytometric analysis of murine mesenteric lymph node cells obtained 3 and 7 days following intraperitoneal injection of TMCF fraction 7. By comparison with control cells, the marked increase in IL-2R-bearing cells 3 days after injection shows that fraction 7 is a rapid activator of IL-2R expression.

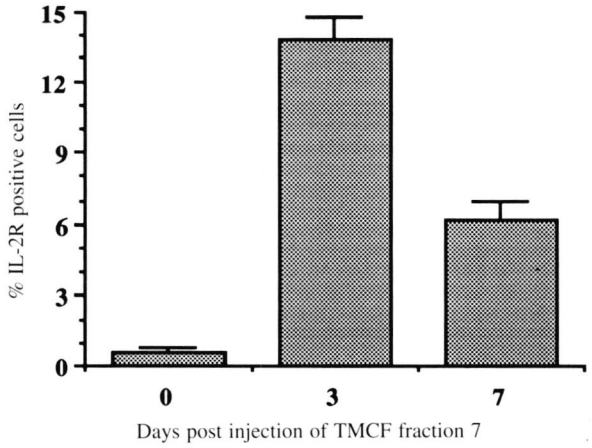


Fig. 1. — Flow cytometric assay of the effect of intraperitoneal injection of TMCF fraction 7 on expression of IL-2 receptor by BALB/c murine mesenteric lymph node cells.

### Lymphocyte expression of FcR and C3b receptor: Effect of TMCF fraction 7

Following intraperitoneal TMCF fraction 7 injection on days 0 and 7, a significant decrease was shown at day 10 in the number of lymphocytes expressing FcR ($\chi^2 > 19.9$; $p < 0.001$). No significant effect was induced by concurrent TMCF injection. No significant change in C3bR expression was detected after TMCF or TMCF fraction 7 injection (Fig. 2).

### T-cell response to phytohaemagglutinin: Enhancement by TMCF fraction 7

The ability of fraction 7 to expand the IL-2R+ population of lymph-node lymphocytes predicts that this fraction should enhance responses to T-cell mitogens. This was tested by subjecting mesenteric lymph-node cells to phytohaemagglutinin stimulation *in vitro* either with or without prior stimulus by intraperitoneal injection of fraction 7. $^3$H-thymidine incorporation (Fig. 3)

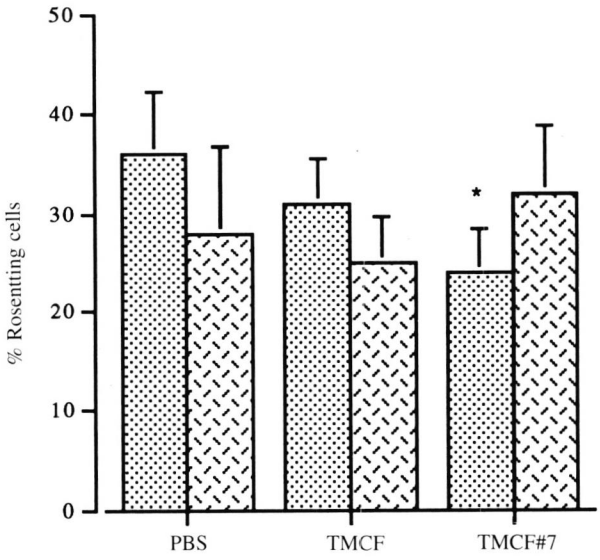


Fig. 2. — Rosetting assay for BALB/c, murine mesenteric lymph-node lymphocytes expressing Fc receptor and C3b receptor 3 days after, double intraperitoneal injection of TMCF or TMCF fraction 7. Mean + s.d. of 3 assays. * $p < 0.001$. ▒ Fc receptor, ▨ C3b receptor.

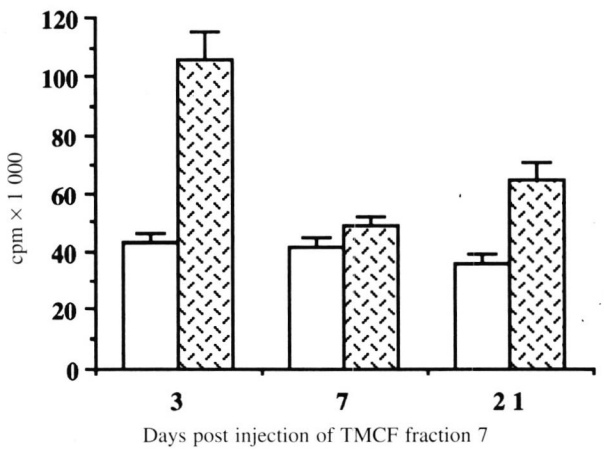


Fig. 3. — $^3$H-thymidine uptake by phytohaemagglutinin stimulated BALB/c, murine mesenteric lymph-node lymphocytes, □ normal lymphocytes; ▨ lymphocytes after intraperitoneal injection of TMCF fraction 7.

showed that fraction 7-treated cells were significantly more reactive to phytohaemagglutinin at 3, 7 and 21 days post injection [Mann-Whitney test at each time interval: $n = 4$, U = 0, $p = 0.05$], indicating a potential of fraction 7 for enhancing this T-cell response.

### T-cell response to ConA: Antagonism between TMCF fraction 7 and fraction 24

Since the action of fraction 7 is accessory cell-dependent (Rakha *et al.*, 1991*b*, 1996), fraction 24 should also

antagonise the mitogenic effects of fraction 7. Figure 4A shows that co-stimulation of normal BALB/c lymph node cells with ConA and fraction 7 led to markedly enhanced mitotic response. Fraction 24 antagonised both the response to ConA and the response to a composite stimulus from ConA and fraction 7. A second assay (Fig. 4B) performed without ConA showed that fraction 7 exerted its usual mitogenic effect on normal lymph node cells. This effect was substantially antagonised by fraction 24.

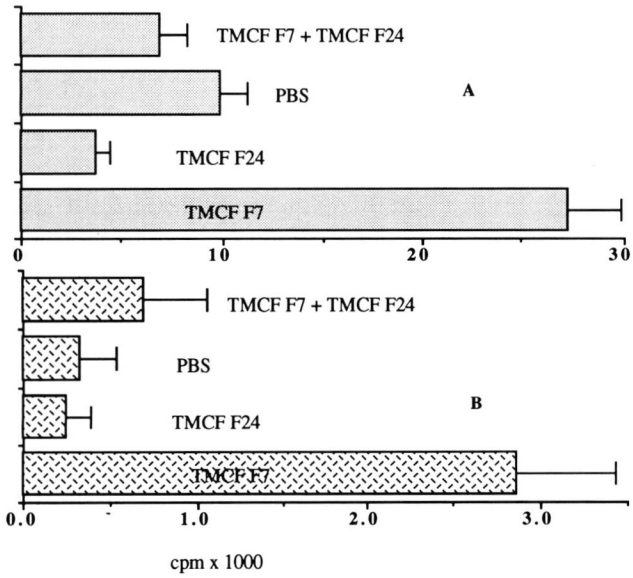


Fig. 4. — $^3$H-thymidine uptake by normal BALB/c, murine mesenteric lymph node cells incubated with 10% TMCF fraction 7, PBS, TMCF fraction 24 or equal parts of fraction 7 and 24. A: Incubation with 3 µg/ml Con A, B: incubation without ConA.

## MACROPHAGE EXPRESSION OF IA: ENHANCEMENT BY TMCF FRACTION 7

Modification of accessory cells has previously been associated with FPLC fraction 24 of *T. multiceps* secretions. It was therefore of interest to find out whether fraction 7 had an effect on the Ia+ population of the macrophages. Figure 5 shows that culture of peritoneal cells with TMCF fraction 7 at 10 % by volume increased the proportion of Ia+ cells in the population at 7 days of incubation [$p$ (two-tailed) <0.05].

## PERITONEAL MACROPHAGE COUNT: ENHANCEMENT BY TMCF FRACTION 7

Three days after a double injection of TMCF fraction 7 into five mice, the number of peritoneal cells recovered per mouse was [6.1 ± 0.6] × $10^6$ as compared to [3.3 ± 0.2] × $10^6$ for five PBS-injected controls ($p < 0.001$). There was concurrent significant rise in the number of plastic-adherent peritoneal cells

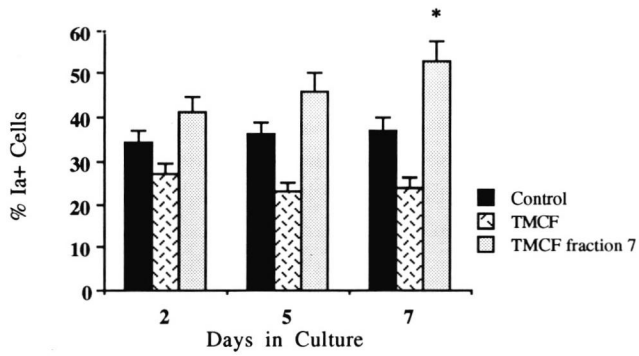


Fig. 5. — Flow cytometric analysis showing percentage of murine Ia+ peritoneal macrophages following incubation *in vitro* with *T. multiceps* cyst fluid or with TMCF-fraction 7 (10 %) (* significant increase with F-7, $p < 0.05$).

($p < 0.01$) and in the proportion of cells which developed cytoplasmic processes in culture ($p < 0.05$). By the seventh day after injection, peritoneal cells from fraction 7-injected mice showed significantly higher viability than those from controls ($p < 0.05$) (results not shown).

## MACROPHAGE EXPRESSION OF FC RECEPTOR: EFFECT OF TMCF AND TMCF FRACTION 7

Figure 6 shows the percentage of peritoneal macrophages forming rosettes with rabbit-IgG-bound sheep red cells. A significant increase in FcR-dependent rosetting was induced by TMCF ($\chi^2 > 6.9$, $p < 0.01$) and TMCF fraction 7 ($\chi^2 > 68.3$, $p < 0.001$) 3 days after the second of two injections.

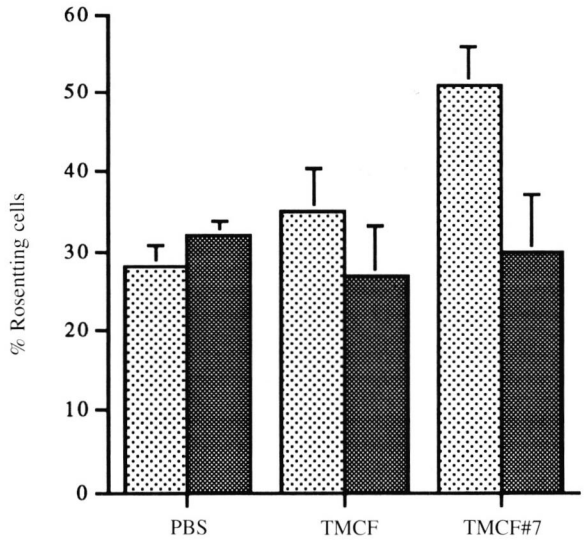


Fig. 6. — Rosette formation by murine macrophages via ▨ FcR or ■ C3bR 3 days after 2 injections of PBS, TMCF or TMCF fraction 7. Mean + s.d. of three experiments.

### Macrophage expression of C3b receptor: Effect of TMCF and TMCF fraction 7

Figure 6 also shows the rosette formation *via* the C3b receptor in macrophages from the same experiment. There was a slight but significant decline in C3bR-dependent rosetting following two TMCF injections ($p < 0.05$).

### Macrophage phagocytosis *via* Fc and C3b: Effect of TMCF and TMCF fraction 7

Figure 7 shows that TMCF enhanced significantly ($p < 0.01$) the percentage of macrophages exhibiting FcR-mediated immunophagocytosis. TMCF fraction 7 caused a larger, more significant enhancement ($p < 0.001$). Neither TMCF nor TMCF fraction 7 significantly changed the number of macrophages phagocytosing *via* C3bR ($p > 0.05$).

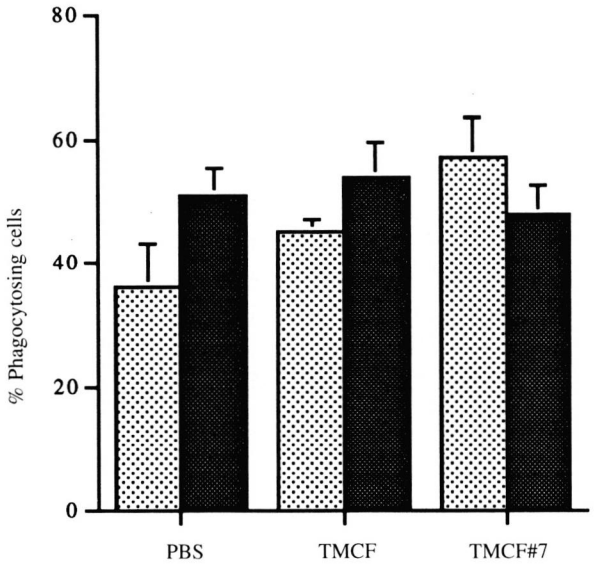


Fig. 7. — Phagocytosis by murine peritoneal macrophages *via* ▨ FcR or ■ C3bR 3 days after a double intraperitoneal injection of PBS, TMCF or TMCF fraction 7. Mean + s.d. of 3 experiments.

### Susceptibility of TMCF fraction 7 to enzymic digestion

Fraction 7 was incubated with protease, trypsin, amylase, neuraminidase, and combinations of these enzymes. When the mitogenic effect of the incubate was tested in ovine peripheral blood lymphocyte cultures, no single enzyme proved capable of destroying fraction 7 activity, but trypsin followed either by amylase or neuraminidase had a deleterious effect on the mitogen. Protease followed by amylase completely destroyed mitogenicity for sheep lymphocytes. Three further experiments produced similar results. Simultaneous treatment of the lymphocytes with fraction 7 together with 30, 40 or 50 µl of the enzymes failed to affect lymphoproliferation. 60 µl had a slightly deleterious effect, probably owing to dilution of TCM by this volume. Thus the enzymes tested were not themselves inhibitory for lymphocyte mitosis (data not shown). It appeared from this that destruction of fraction 7 could be effected only by attack on both peptide and carbohydrate moieties.

## DISCUSSION

The mitogenic action of FPLC fraction 7 of *T. multiceps* coenurus fluid (TMCF) was not affected by the lipopolysaccharide antagonist, polymixin B, and was not associated with detectable endotoxin. These results are in accordance with those of Sealey *et al.* (1981) working with *T. solium*. These authors, the first to draw attention to the mitogenic potential of taeniid secretions, also found the effect to be independent of endotoxin lipopolysaccharide. Together these results indicate that non-specific mitosis of T cells is probably induced by metacestode-derived factors.

As to the mode of action of the *T. multiceps* mitogen, the fact that TMCF fraction 7 proved capable of increasing the IL-2 receptor-bearing population of lymph nodes implies that the factor could by this means act as a second signal to enhance lymphoproliferation. The enhancement of phytohaemagglutinin and ConA-induced proliferation by fraction 7 is consistent with this interpretation. At the same time, this does not preclude the possibility that mitogenic stimuli from metacestodes could also provide a primary signal for mitosis. Thus the mitogenic activities in *T. multiceps* and *Echinococcus granulosus* are macrophage-dependent and class II restricted (Rakha *et al.*, 1991*b*; Cox *et al.*, 1986), the latter finding suggesting the involvement of antigen receptor in the mitotic signal, despite the fact that naive T cells respond readily to the stimulus. The hypothesis that the parasite-derived molecules act as superantigens is currently being investigated. The biological significance of non-specific lymphoproliferation is not yet clear. The effect may promote responses to a number of antigens (Rakha *et al.*, 1991*b*). On the other hand, non-specific T-cell proliferation also has the potential to expand CD8+ T-cell populations with suppressor activity (Kizaki *et al.*, 1993*a, b, c*).

The decrease in the lymph-node cell population bearing Fc receptor for IgG can be attributed to the expansion of the T-cell component of mesenteric lymph nodes under the action of fraction 7. Injection of TMCF fraction 7 resulted in significant increases in peritoneal macrophage Fc receptor expression as well

as in count, viability and formation of cytoplasmic processes. The rosetting method used here does not distinguish between types of Fc receptor. In any case, murine Fc receptors are probably not entirely isotype specific (Unkeless *et al.,* 1988; Takizawa *et al,* 1992). Consequently one cannot predict in detail the effect of altered Fc receptor expression on macrophage behaviour. Nevertheless, fraction 7-modified macrophages displayed an increased rate of Fc-mediated phagocytosis. Enhanced receptor expression under the influence of fraction 7 would therefore be calculated to improve the efficiency of macrophages as parasiticidal effector cells. In accordance with this, the normal killing power of peritoneal macrophages for *E. multilocularis* protoscoleces is increased by infection and by the presence of specific antisera (Rau & Tanner, 1976; Baron & Tanner, 1977). Changes in the expression of C3b receptor on macrophages were, by comparison, slight, and were not associated with altered C3b-mediated phagocytosis.

Fraction 7-modified macrophages showed an increased proportion of cells expressing Ia antigen. This finding is consistent with a previous report (Rakha *et al.,* 1991*b*) that such modified macrophages are improved accessory cells for ConA-stimulated T-cell proliferation. By contrast, fraction 24 of TMCF is a proven inhibitor of Ia expression and of accessory function in macrophages. Thus with induction of macrophage Ia expression, as with induction of antigen-specific lymphocytes, fractions 7 and 24 have opposite regulatory actions (Rakha *et al.,* 1991*b*, 1996). This versatile activity could give the parasite considerable influence over the local immune response. Parasite-induced effects like inhibition of accessory function are easily interpretable as immune evasion. Others like enhanced phagocytosis are, at first sight, incompatible with the parasite's interests. Nevertheless, there are examples of chronic parasites, e.g. *Trypanosoma cruzi,* producing secretions which, paradoxically, limit parasite virulence. This may be part of a ploy to protect the host from extremes of parasite aggression and to preserve the essential balance of host-paraite relationship (Pereira, 1988). While the details of metacestode immunoregulation require much future study, the importance of the process is suggested by the fact that hydatids have similar chromatographic fractions with similar immunological activities to those of *T. multiceps* (Dixon & Jenkins, 1995*b*). In hydatidosis in particular, understanding the function of these potential immunoregulators may indicate a rationale for immunotherapy.

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