mole percentages of the bases were determined from the area of the peaks in the elution pattern compared with the areas of calibration curves made by using pure bases as standards.

Results and discussion. The agarose gel electrophoresis of the restricted MaDNA is shown in figure 1. The two restriction endonucleases used, Hpa II and Msp I, both recognize the sequence 5'-CCGG but while Hpa II does not cleave when internal cytosine is methylated, Msp I cleaves the sequence regardless of methylation. Msp I digestion of MaDNA produces shorter fragments than Hpa II digestion. The fact that Msp I is more active than Hpa II points to the presence of MeCyt in the *Blepharisma japonicum* genome.

This result is confirmed by HPLC analysis (fig. 2). A clear peak occupying the same position of standard MeCyt is seen after 12 min elution from sample injection. The adenine retention time is 20.2 min not shown in figure (see Citti et al.[19]). The MeCyt content corresponds to about 0.56 mole per cent of the total cytosine; this amount comes from the averaged value of four replicated analyses of two distinct MaDNA extractions. The minor peaks present immediately after injection represent other minor bases not yet well identified and partially hydrolyzed DNA (unpublished results). This fact may cause an underestimate of the amount of MeCyt in MaDNA.

The importance of this short report resides in the demonstration of the presence of MeCyt in the MaDNA of a ciliate protist. The MaDNA from all ciliates examined up to now has only been found to contain MeAde. Our finding contrasts *Blepharisma japonicum* with other ciliates but it aligns this species with the other unicellular autotrophic eukaryotes that make use of cytosine methylation like *Euglena*[20], *Chlamydomonas*[12], and *Exuviella*[9]. In prokaryotes both adenine and cytosine can be methylated, whereas in multicellular organisms MeCyt is the most widespread or the unique modified base. Among ciliates, considered to be the most animal-like of all protists, the methylation of cytosine residues seems to have disappeared in many species, but it persists in *Blepharisma*.

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The effect of mouse erythrocyte rosette forming lymphocytes on lymphokine production in T-cell cultures

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Summary. An enhancement effect of mouse erythrocyte rosette forming (MERF) cells on the production of migration inhibitory factor, chemotactic factor for neutrophils and skin reactive factor in T-lymphocyte cultures stimulated with the purified protein derivative of tuberculin was observed. We consider it likely that the MERF cells, possessing the appropriate cell surface constituents to construct an immunogenic moiety, present antigen on their surfaces to elicit lymphokine production.
Key words. Lymphokines; mouse erythrocyte binding lymphocytes; T-lymphocytes.

Lymphocytes stimulated by a mitogen or a specific antigen produce a number of soluble factors (lymphokines) with various activities. These include macrophage migration inhibitory factor (MIF), chemotactic factor for neutrophils (CFN), skin reactive factor (SRF), etc. These factors play a part in the inflammatory processes of delayed-type hypersensitivity. Both T- and B-cells are able to produce lymphokines[2,3], but the interactions between these two populations of lymphocytes in lymphokine synthesis have been studies less extensively.

Lymphocytes forming rosettes with mouse erythrocytes (MERF cells) have been detected in the peripheral blood. The MERF lymphocytes proved to be B-cells which have surface immunoglobulin and receptors for C3 which do not possess T-cell specific markers[4,5]. Rosette formation with mouse erythrocytes (ME) seems to define a population unresponsive to pokeweed mitogen and to be a marker for resting B-lymphocytes[6]. ME rosette-positive B-cells stimulate poorly in autologous and allogenic mixed lymphocyte reactions[7].

The aim of this investigation was to study the effect of MERF cells on the production of MIF, CFN and SRF by T-lymphocytes stimulated with a purified protein derivative of tuberculin (PPD), and thus to obtain data on the role of this B-cell subpopulation in T- and B-lymphocyte interactions.

Methods. Samples of blood were collected from 31 PPD skin test-positive healthy volunteers. Peripheral blood mononuclear cells (PBMC) were separated by density gradient centrifugation, using the method described by Böyum[8].

T-lymphocytes were isolated from a PBMC suspension by a positive selection method. Aliquots of PBMC partially depleted of adherent cells, and sheep red blood cells (SRBC) were mixed at a final ratio of SRBC to PBMC of 20:1, centrifuged for 3 min at 400 g at room temperature, and then incubated

The effect of MERF cells on lymphokine production in T-cell cultures

Lymphokine activity[a]	n	Cultures containing[a] 10^6 PBMC/ml	10^6 T-cells/ml	10^6 T-cells and 5×10^4 MERF cells/ml	10^6 T-cells and 10^5 MERF cells/ml	10^5 MERF cells/ml	Control cultures[a]
MIF activity	12	0.61 ± 0.11	0.83 ± 0.07[b]	0.70 ± 0.04[c]	0.67 ± 0.05	0.97 ± 0.08	0.95 ± 0.03
CFN activity	13	81.2 ± 17.2	20.3 ± 10.1[b]	32.9 ± 8.4[c]	31.1 ± 14.6[c]	7.3 ± 4.7	5.0 ± 3.7
SRF activity	6	7.6 ± 1.17	3.9 ± 1.06[b]	5.74 ± 0.91[c]	6.2 ± 0.71[c]	2.03 ± 0.31	1.61 ± 0.93

Data given as mean ± SD; p was calculated by Student's paired t-test. [a] See 'Methods'. [b] Significantly lower activity than in cultures containing 10^6 PBMC/ml. [c] Significantly higher activity than in cultures containing 10^6 T-cells/ml.

for 2 h at 4 °C. Subsequently, the sediment was suspended by careful rotation, and the resuspended mixture was underlayered with a Ficoll-Uromiro gradient and centrifuged at 1000 × g for 20 min at 4 °C. After centrifugation, the E rosette-forming T-lymphocytes were enriched in the sediment. SRBC present in pelleted cells were lysed with buffered ammonium chloride. The collected T-lymphocytes were washed three times. T-cells were 95% E rosette$^+$, 2% surface immunoglobulin$^+$, 0.5% esterase$^+$ and 2% Ia$^+$.

MERF lymphocytes were isolated in a similar way, as described earlier[9]. The T-cell contamination was eliminated by treatment with T-cell specific serum and complement. (Polyclonal anti-T-cell anti-serum was given by Dr Aftab Achmed, Dept. of Immunology, Naval Medical Research Institute, Bethesda, Maryland). MERF cells were 91% surface immunoglobulin$^+$, 3% E rosette$^+$, 0.5% esterase$^+$ and 64% Ia$^+$.

From each donor, 6 types of culture were prepared in 3 ml Parker 199 medium supplemented with L-glutamine and 10% heat-inactivated fetal calf serum. The first contained 10^6 PBMC/ml, the second 10^6 T-lymphocytes/ml, the third 10^6 T-lymphocytes and 5×10^4 MERF cells/ml, the fourth 10^6 T-lymphocytes and 10^5 MERF cells/ml, and the fifth 10^5 MERF cells/ml. These cultures were stimulated with 1 µg PPD/ml. The stimulated cultures were incubated at 37 °C for 24 h and then centrifuged, and the lymphokine activities of the supernatants were examined. The sixth culture, which served as control, contained 10^6 T-lymphocytes/ml and the PPD was added after the 24 h in culture.

MIF activity was measured in triplicate cultures by a conventional in vitro assay, using guinea pig peritoneal exudate cells packed in capillary tubes[10]. CFN activity was detected in a Boyden chamber, using 10^7 granulocytes/ml[11]; the activity was tested in duplicate. In the measurement of SRF activity, undiluted supernatants were drawn into tuberculin syringes and each of them was injected intradermally into 3 PPD skin test-negative guinea-pigs. The diameter of the erythematous skin reaction was examined 10 h after the injection[12].

Results. The results are summarized in the table. The positively selected T-lymphocytes produced significantly less lymphokine than did the unfractionated PBMC cultures. In every case, lower MIF, CFN und SRF activities were measured in the T-cell cultures than in the PBMC cultures of the same donor.

The lymphokine production of the T-lymphocytes was enhanced significantly if 5–10% MERF cells were added to the cultures. However, the MIF, CFN and SRF activities of the cultures also containing MERF cells did not attain those of the PBMC cultures.

PPD did not induce measureable extents of MIF, CFN or SRF production in cultures containing 10^5 MERF cells/ml.

Discussion. An earlier report established that leukocyte migration inhibitory factor (LIF) production in response to PPD was restricted to T-lymphocytes[13]. It was later concluded that LIF production is linked to the active E rosette-forming cells[14]. In the present work it was found that the positively selected T-cells produce MIF, CFN and SRF in considerable amounts, though these levels are significantly lower than those in PBMC cultures.

The addition of 5–10% MERF cells to the T-lymphocyte cultures led to a significant enhancement of the lymphokine production. The higher MIF, CFN and SRF activities cannot be a consequence of a possible lymphokine formation from the MERF cells because mediator activity could not be detected in cultures containing 10^5 MERF cells/ml. This indicates that the higher lymphokine activity observed in cultures containing both T- and B-cells is a consequence of cooperation between the two populations. Since rosette formation with ME seems to define a resting subpopulation of B-lymphocytes, we do not consider it very probable that this enhancing effect is a result of an active metabolic interaction.

It is generally thought that macrophages present antigen on their surfaces to elicit T-cell-mediated functions. Several studies suggest that specific physical interactions between antigen-presenting cells and primed responders permit antigen recognition in the context of Ia molecules[15,16]. However, antigen presentation may not be exclusively a function of macrophages. Other Ia$^+$ cells (e.g. B-lymphocytes and active E rosette-forming lymphocytes) may effectively present antigen and thereby induce immune responses[17,18]. We consider it likely that the MERF cells, possessing the appropriate cell surface constituents to construct an immunogenic moiety, present antigen on their surfaces to elicit lymphokine production. These data indicate that the antigen-presenting function of the MERF cells is more effective than that of the active E rosette-forming cells to be found in the T-cell culture.

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