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Cytophilic anti-thyroglobulin antibody and antibody-dependent macrophage-mediated cytotoxicity in Hashimoto's thyroiditis.

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## Abstract

Antibody-dependent macrophage-mediated cytotoxicity was studied to determine the significance of cytophilic anti-thyroglobulin antibody (ATgA) present in the sera of patients with Hashimoto's thyroiditis. Effector cells were normal human monocytes or guinea-pig peritoneal exudate cells, and target cells were human thyroglobulin(Tg)-coated chicken erythrocytes. Cytotoxicity was evaluated by morphological observation and by <sup>51</sup>Cr-releasing assay. Normal human monocytes rapidly destroyed ATgA-bound Tg-coated chicken erythrocytes by extracellular cytolysis and by phagocytosis. On the contrary, human monocytes "armed" with cytophilic ATgA destroyed Tg-coated chicken erythrocytes slowly and to a lesser extent, and only by extracellular cytolysis. When normal monocytes or peritoneal exudate cells were incubated with Tg-coated chicken erythrocytes in the presence of the sera of patients with Hashimoto's thyroiditis, phagocytosis occurred rapidly, but extracellular cytolysis developed rather slowly. These data suggest the possibility that human monocytes participate in antibody-dependent cell-mediated cytotoxicity (ADCC) in vivo, which may be an important destructive mechanism in Hashimoto's thyroiditis. It is also possible that ATgA cytophilic for monocytes render non-immune peripheral monocytes cytotoxic against Tg-bearing cells.

**KEYWORDS:** antibody-dependent cell-mediated cytotoxicity, cytophilic antibody, Hashimoto's thyroiditis, antibody-dependent macrophage-mediated cytotoxicity

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## Cytophilic Anti-thyroglobulin Antibody and Antibody-dependent Macrophage-mediated Cytotoxicity in Hashimoto's Thyroiditis

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Antibody-dependent macrophage-mediated cytotoxicity was studied to determine the significance of cytophilic anti-thyroglobulin antibody (ATgA) present in the sera of patients with Hashimoto's thyroiditis. Effector cells were normal human monocytes or guinea-pig peritoneal exudate cells, and target cells were human thyroglobulin(Tg)-coated chicken erythrocytes. Cytotoxicity was evaluated by morphological observation and by <sup>51</sup>Cr-releasing assay. Normal human monocytes rapidly destroyed ATgA-bound Tg-coated chicken erythrocytes by extracellular cytolysis and by phagocytosis. On the contrary, human monocytes "armed" with cytophilic ATgA destroyed Tg-coated chicken erythrocytes slowly and to a lesser extent, and only by extracellular cytolysis. When normal monocytes or peritoneal exudate cells were incubated with Tg-coated chicken erythrocytes in the presence of the sera of patients with Hashimoto's thyroiditis, phagocytosis occurred rapidly, but extracellular cytolysis developed rather slowly. These data suggest the possibility that human monocytes participate in antibody-dependent cell-mediated cytotoxicity (ADCC) *in vivo*, which may be an important destructive mechanism in Hashimoto's thyroiditis. It is also possible that ATgA cytophilic for monocytes render non-immune peripheral monocytes cytotoxic against Tg-bearing cells.

*Key words* : antibody-dependent cell-mediated cytotoxicity, cytophilic antibody, Hashimoto's thyroiditis, antibody-dependent macrophage-mediated cytotoxicity

It is well-known that monocytes/macrophages play important roles in allograft rejection (1), tumor immunity (2) and auto-immune diseases (3,4). Many investigators have reported that non-immune monocytes/macrophages destroy various target cells such as erythrocytes (5), virus-infected cells (6) and tumor cells (7) in the presence of antibodies specific for the target cells. This destructive mechanism, which was described first by Möller (8), has been called antibody-dependent cell-mediated cytotoxicity (ADCC). Other effector cells responsible for ADCC have been found to include neutro-

phils (9, 10), T cells (11) and so called K cells or null cells (12, 13), all of which bear receptors for the Fc portion of IgG.

In contrast, supernatants from heat-treated or cultured immune effector cells can render non-immune effector cells adherent to or cytotoxic to target cells which are used for immunization. The effective factors contained in the supernatants are known to be cytophilic antibodies (14-17). These antibodies are also present in hyper-immune sera (1, 5, 15), and can "arm" non-immune effector cells via their Fc portion and mediate cytotoxicity against antigen bearing

target cells (18, 19).

In Hashimoto's thyroiditis, infiltration into the thyroid by macrophages, as well as by plasma cells and lymphocytes, is not uncommon. However, it is not clearly known why and by what mechanism these cells infiltrate the thyroid, nor how they work destructively in collaboration with lymphocytes or autoantibodies such as anti-thyroglobulin antibodies (ATgA) or anti-microsomal antibodies (AMcA) present in the sera of patients with Hashimoto's thyroiditis.

In the present study, we assayed macrophage-mediated ADCC, using Tg-coated chicken erythrocytes as target cells, in order to determine the significance of cytophilic antibodies in the mechanism of ADCC in Hashimoto's thyroiditis.

## Materials and Methods

*Patients.* Eleven patients with clinically and/or histologically diagnosed Hashimoto's thyroiditis and eleven patients with Graves' disease were examined. Eight normal subjects were examined as controls.

*Target cells.* Erythrocytes were obtained from heparinized peripheral blood of 1-6 month-old chickens and washed 4 times with sterilized physiological saline. The washed erythrocytes were coated with purified human Tg using chromic chloride as a coupling agent (20). Purification of Tg was performed according to the method of Shulman and Armenia (21). For the  $^{51}\text{Cr}$  releasing assay,  $2 \times 10^7$  Tg-coated chicken erythrocytes in saline containing 100-150  $\mu\text{Ci}$  of sodium [ $^{51}\text{Cr}$ ] chromate (70-120 mCi/mg, Daiichi Radioisotope Laboratories Co., Ltd., Tokyo) were shaken at 37°C for 45 min in a water bath. The cells were washed 4 times with Hanks' balanced salt solution (HBSS) and resuspended in TCM 199 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U/ml of penicillin and 100  $\mu\text{g}/\text{ml}$  of streptomycin (TCM-FCS). Target cells were kept at 0-4°C until used.

*Effector cells.* Lymphoid cells of normal subjects were separated by the Ficoll-Conray 400 gradient method described by Böyum (22), washed 3 times with cold HBSS and resuspended in TCM-FCS. After adjusting cell counts as indicated, aliquots of the suspension were poured into test tubes (12×105 mm) for the  $^{51}\text{Cr}$  releasing assay or onto cover slips placed in Leighton tubes for the morphological study. Incubation was performed at 37°C for 1 h in humidified 5%  $\text{CO}_2$ -air. After the incubation, non-adherent cells were counted and washed away with warm HBSS. The adherent cells were used as effector cells. Numbers of adherent cells were estimated by subtracting the non-adherent cell count from the total cell count. Viability of these cells was ascertained by trypan blue exclusion test. More than 95% of the adherent cells were viable monocytes.

In some experiments, guinea-pig peritoneal exudate cells were used as effector cells. Guinea-pigs weighing 300-500 g were injected intraperitoneally with 10 ml/kg of 5% proteose-peptone (Difco) and 5% starch in saline 3 days before each experiment. On the day of experiments, the animals were bled. Peritoneal exudate cells were obtained by washing peritoneal cavities with HBSS. The cell suspension was washed 3 times with HBSS and resuspended in TCM-FCS. Adherent cells were obtained as described above; over 90% of them were viable macrophages.

*Antisera.* Sera of patients with Hashimoto's thyroiditis or Graves' disease, and of normal subjects were heat-inactivated at 56°C for 30 min. Sera were then incubated with a tenth volume of packed chicken erythrocytes at 37°C for 1 h and left at 0-4°C overnight. The erythrocytes were then removed by centrifugation at 1500×g for 5 min to remove natural anti-chicken erythrocyte antibodies. ATgA titers were evaluated using a commercially available kit (Thyroid test, Fujizoki Pharmaceutical Co., Ltd., Tokyo).

*Sensitization of target cells with antibodies.* About  $1 \times 10^7$  target cells were incubated with 10 to 100 times diluted patient's sera at 37°C for 30 min, washed 3 times with HBSS and resuspended in TCM-FCS.

*Arming of effector cells with antibodies.* Normal effector cells were incubated with sera of patients with Hashimoto's thyroiditis at 0-4°C

for 1 h, then washed more than 4 times with cold HBSS and used as armed effector cells. The arming capability of the sera was confirmed by observing rosette formation of effector cells with Tg-coated chicken erythrocytes *in vitro* (23-25).

**Morphological studies.** Suspensions in TCM-FCS containing  $2 \times 10^7$ /ml of Tg-coated chicken erythrocytes bound or not bound with ATgA were poured onto monolayer cultures of normal or antibody-armed monocytes on cover slips in Leighton tubes. Incubation was performed at 37°C for 1 h to 6 h in humidified 5% CO<sub>2</sub>-air. After the incubation, all cover slips were washed 3 times with warm HBSS, and air-dried. The cells were fixed with methanol, stained with Giemsa and observed under a light microscope. The results were expressed as follows:

$$\% \text{ Binding} = \frac{\text{number of monocytes which bound more than three target cells among 500 monocytes}}{500} \times 100$$

$$\% \text{ Phagocytosis} = \frac{\text{number of monocytes which phagocytized target cells among 500 monocytes}}{500} \times 100$$

**Cytotoxicity assay.** Three different cytotoxicity assays were performed: (a) cytotoxicity of normal monocytes against ATgA-bound Tg-coated chicken erythrocytes (b) cytotoxicity of antibody-armed monocytes against Tg-coated chicken erythrocytes and (c) cytotoxicity of normal monocytes in the presence of antibodies against target cells. About  $5 \times 10^5$  effector cells (normal or armed) were incubated at 37°C for 8 h to 24 h, in a final volume of one ml, with  $1 \times 10^5$  target cells (Tg-coated chicken erythrocytes bound or not bound with ATgA) in the presence or absence of the sera of patients with Hashimoto's thyroiditis. After the incubation, the incubation mixture was centrifuged at  $200 \times g$  for 10 min. Total and supernatant radioactivity in each tube was measured with a well-type gamma scintillation counter. The cytotoxicity was expressed as follows:

$$\text{net } \% \text{ } ^{51}\text{Cr release} = \frac{A-C}{B-C} \times 100,$$

where A is the radioactivity of the supernatant of the reaction mixture with effector cells and patient's serum, B is the total radioactivity and

C is the spontaneous radioactivity, *i. e.*, the supernatant radioactivity of the reaction mixture without effectors or patient's serum.

**Phagocytosis assay.** After the radioactivity determination for net % <sup>51</sup>Cr release as described above, the supernatant was carefully aspirated out, and 1 ml of distilled water was added to lyse non-phagocytized erythrocytes. After a few minutes, the mixture was centrifuged at  $200 \times g$  for 10 min. The radioactivity in the sediment, which indicates phagocytized radioactivity, was measured. The extent of phagocytosis was calculated by the following equations:

$$\% \text{ phagocytosis} = \frac{D}{B} \times 100,$$

where D is phagocytized radioactivity and B is total radioactivity, and

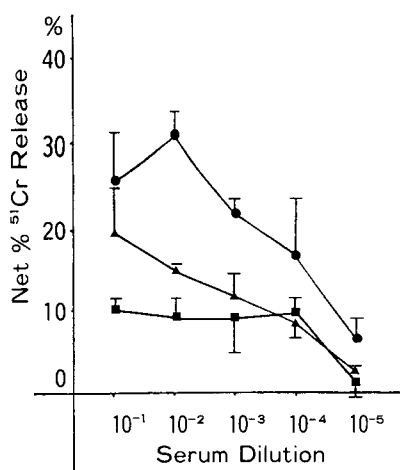
$$\begin{aligned} \text{net } \% \text{ phagocytosis} = \\ \% \text{ phagocytosis of each experiment} - \\ \% \text{ phagocytosis in experiments without} \\ \text{monocytes or patient's serum} \end{aligned}$$

**Blocking study.** In order to examine the blocking effect of non-specific or unrelated IgG in sera on monocyte-mediated cytolysis, the following experiments were performed. Normal monocytes were incubated at 37°C for 4 h with ATgA-bound Tg-coated chicken erythrocytes, and armed monocytes were incubated at 37°C for 8 h with Tg-coated chicken erythrocytes. Each experiment was performed in the presence of normal sera (final dilution, 1 : 20, 1 : 100 and 1 : 200) or Cohn's fraction II (Sigma) (1 μg, 10 μg, and 100 μg/ml).

All cultures were performed in triplicate, and cytotoxicity was expressed as the mean ± S. D.

## Results

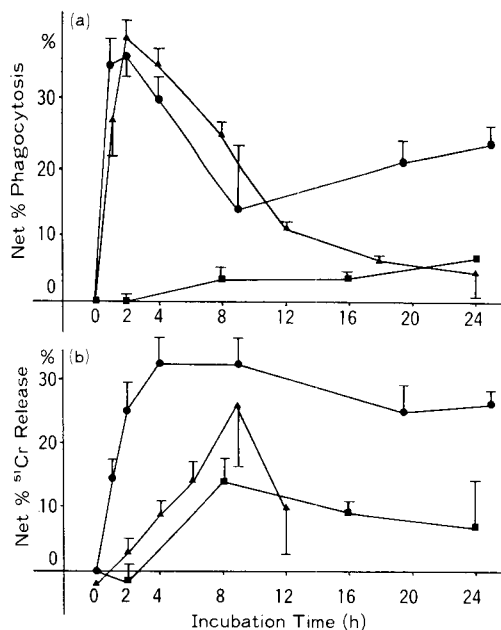
*The cytotoxicity of normal monocytes against Tg-coated chicken erythrocytes in the presence of sera of patients with Hashimoto's thyroiditis.* Significant cytotoxicity was observed under the following optimal conditions: the effector to target cell ratio of 1-3 : 1 and a final serum dilution of 1 : 10-1 : 1000 (Fig. 1). As can be seen in Fig. 2, net % <sup>51</sup>Cr release increased



**Fig. 1** Effect of serum dilution on cytotoxicity. ●—●, Cytotoxicity of normal monocytes against ATgA-bound Tg-coated chicken erythrocytes (incubation, 4 h, effector to target cell ratio, 4 : 1); ▲—▲, cytotoxicity of normal monocytes against Tg-coated chicken erythrocytes in the presence of sera of patients with Hashimoto's thyroiditis (incubation, 6 h, effector to target cell ratio, 2 : 1); ■—■, cytotoxicity of monocytes armed with sera of patients with Hashimoto's thyroiditis against Tg-coated chicken erythrocytes (incubation, 8 h; effector to target cell ratio, 5 : 1).

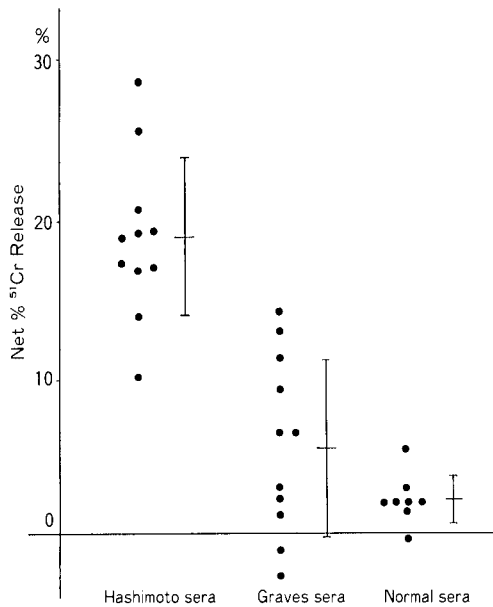
slowly, while net % phagocytosis increased rapidly and markedly. After 4 h of incubation, net % phagocytosis decreased gradually. Net % <sup>51</sup>Cr release was significantly higher in the presence of sera of patients with Hashimoto's thyroiditis than in the presence of normal sera or sera of patients with Graves' disease (Fig. 3). Cytotoxicity correlated closely with the logarithm of the ATgA titer (Fig. 4).

*The cytotoxicity of peritoneal exudate cells against Tg-coated chicken erythrocytes in the presence of sera of patients with Hashimoto's thyroiditis.* As shown in Fig. 5, net % <sup>51</sup>Cr release increased gradually with time. On the other hand, net % phagocytosis increased rapidly and markedly up to 2 h of incubation, and then decreased gradually. These data were quite similar to those obtained from experiments using human monocytes as described above.

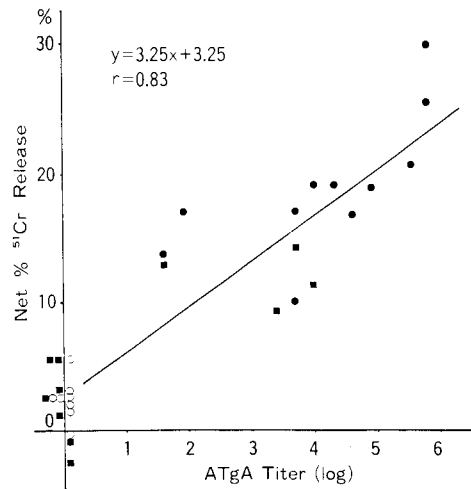


**Fig. 2** Time course of (a) phagocytosis and (b) cytotoxicity. ●—●, Cytotoxicity and phagocytosis of normal monocytes incubated with ATgA-bound Tg-coated chicken erythrocytes (effector to target cell ratio, 5 : 1; sera dilution for sensitization, 1 : 1000); ▲—▲, cytotoxicity and phagocytosis of normal monocytes incubated with Tg-coated chicken erythrocytes in the presence of sera of patients with Hashimoto's thyroiditis (final serum dilution, 1 : 1000; effector to target cell ratio, 5 : 1); ■—■, cytotoxicity and phagocytosis of monocytes armed with sera of patients with Hashimoto's thyroiditis (1 : 100 dilution) incubated with Tg-coated chicken erythrocytes (effector to target cell ratio, 5 : 1).

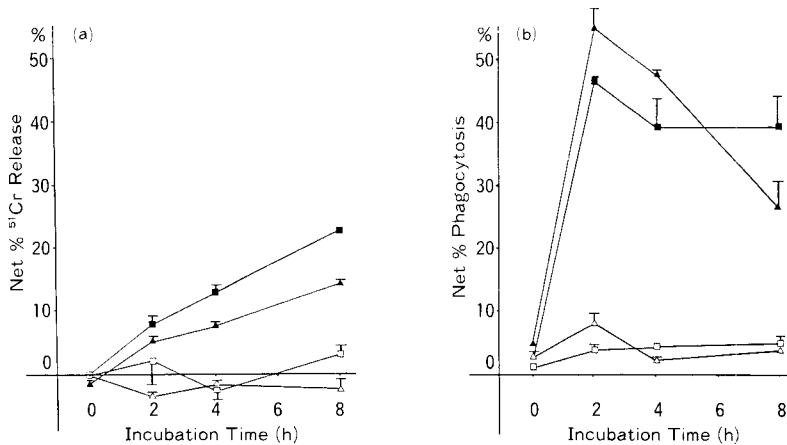
*The cytotoxicity of normal monocytes against Tg-coated chicken erythrocytes treated with sera of patients with Hashimoto's thyroiditis.* Similar to the above mentioned experiments, significant cytotoxicity was observed in the system using normal human monocytes. As can be seen in Fig. 2, a large percent of <sup>51</sup>Cr was released from the antibody-coated target cells within 2-4 h. Thereafter, net % <sup>51</sup>Cr release reached a plateau ( $32.8 \pm 4.1\%$ ), while net % phagocytosis, which also increased rapidly until reaching a plateau, decreased gradually thereafter. Morphological observations showed that a significantly high level of %



**Fig. 3** Cytotoxicity of normal monocytes against Tg-coated chicken erythrocytes in the presence of sera of patients with Hashimoto's thyroiditis or Graves' disease, and of normal subjects. Incubation time, 6 h. Effector to target cell ratio, 3 : 1. Final serum dilution, 1 : 500.



**Fig. 4** The correlation between cytotoxicity and ATgA titer. Incubation time, 6 h. Effector to target cell ratio, 3 : 1. Final serum dilution, 1 : 500. ●, Cytotoxicity of normal monocytes against Tg-coated chicken erythrocytes in the presence of sera of patients with Hashimoto's thyroiditis; ■, cytotoxicity of normal monocytes against Tg-coated chicken erythrocytes in the presence of sera of patients with Graves' disease; ○, cytotoxicity of normal monocytes against Tg-coated chicken erythrocytes in the presence of normal sera.



**Fig. 5** Cytotoxicity of normal or armed guinea-pig peritoneal exudate cells against Tg-coated chicken erythrocytes in the presence or absence of sera of patients with Hashimoto's thyroiditis. Cytotoxicity was evaluated by (a) <sup>51</sup>Cr release assay and (b) phagocytosis of target cells. ▲—▲, Cytotoxicity of normal peritoneal exudate cells against Tg-coated chicken erythrocytes in the presence of sera of patients with Hashimoto's thyroiditis (final serum dilution, 1 : 1000; effector to target cell ratio, 5 : 1); △—△, cytotoxicity of normal peritoneal exudate cells incubated with Tg-coated chicken erythrocytes in the presence of normal human sera (final serum dilution, 1 : 1000; effector to target cell ratio, 5 : 1); ■—■, cytotoxicity of peritoneal exudate cells armed with sera of patients with Hashimoto's thyroiditis (dilution, 1 : 100) against Tg-coated chicken erythrocytes (effector to target cell ratio, 5 : 1); □—□, cytotoxicity of peritoneal exudate cells armed with normal human sera (dilution, 1 : 100) against Tg-coated chicken erythrocytes (effector to target cell ratio, 5 : 1).

binding was reached at 1 h (16.0%), but the level decreased after 3 h of incubation, while % phagocytosis increased rapidly and markedly at 1 h (70.2%) and did not decrease even after 3 h of incubation (Table 1).

*The cytotoxicity of armed monocytes against Tg-coated chicken erythrocytes.* Significant cytotoxicity of armed monocytes was observed under the optimal conditions described above. However, the degree of the maximum net %  $^{51}\text{Cr}$  release was not as

**Table 1** Morphological evaluation of the reaction between normal or armed monocytes and Tg-coated chicken erythrocytes or anti-Tg antibody bound Tg-coated chicken erythrocytes

Effector cells + Target cells	% Binding <sup>a</sup>			% Phagocytosis <sup>b</sup>		
	Incubation time (h)			Incubation time (h)		
	1	3	6	1	3	6
Normal monocytes + CE-Tg <sup>c</sup>	0.0	0.0	0.6	0.0	0.0	0.0
Armed monocytes <sup>d</sup> + CE-Tg <sup>c</sup>	31.0	30.0	8.4	0.4	3.2	9.8
Normal monocytes + CE-Tg-Ab <sup>e</sup>	16.0	3.8	n. d. <sup>f</sup>	70.2	74.8	n. d. <sup>f</sup>
Armed monocytes <sup>d</sup> + CE-Tg-Ab <sup>e</sup>	50.2	n. d. <sup>f</sup>	n. d. <sup>f</sup>	47.6	n. d. <sup>f</sup>	n. d. <sup>f</sup>

a: Percentage of normal or anti-thyroglobulin antibody-armed monocytes which bound target cells (500 monocytes were counted).

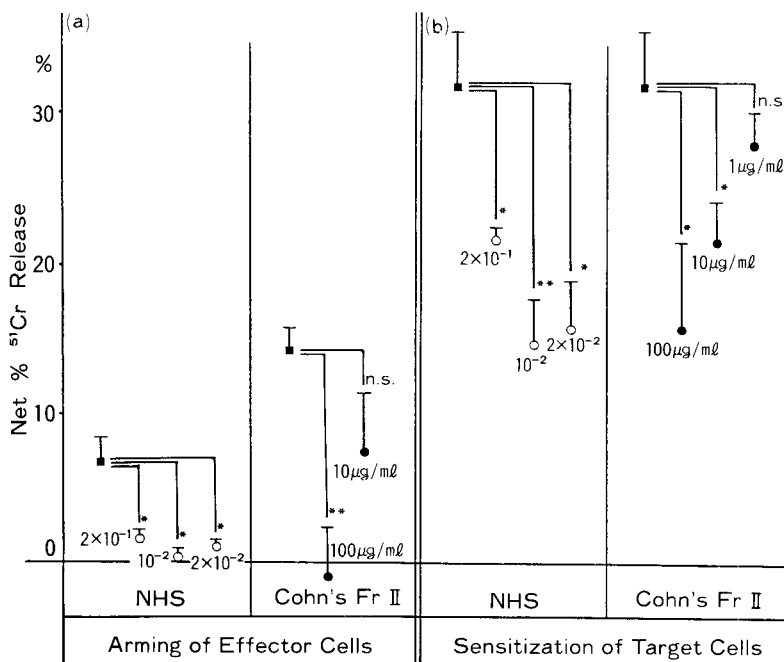
b: Percentage of normal monocytes which phagocytized target cells (500 monocytes were counted).

c: Human thyroglobulin-coated chicken erythrocytes.

d: Anti-thyroglobulin antibody bound monocytes.

e: Anti-thyroglobulin antibody bound thyroglobulin-coated chicken erythrocytes.

f: Not done.



**Fig. 6** Blocking effect of normal human sera (NHS) or Cohn's fraction II (Fr II) on monocyte cytotoxicity. (a) Monocytes armed with sera of patients with Hashimoto's thyroiditis (dilution, 1 : 100) were incubated for 8 h with Tg-coated chicken erythrocytes (effector to target cell ratio, 5 : 1). (b) Normal monocytes were incubated for 4 h with ATgA-bound Tg-coated chicken erythrocytes (dilution of sera for sensitization, 1 : 400, effector to target cell ratio, 5 : 1). Statistical evaluation was performed by Student's *t* test; \*\*  $p < 0.01$  \*  $p < 0.05$ .



high ( $14.2 \pm 3.7\%$ ) as when normal monocytes were assayed (Fig. 2), and no significant phagocytosis occurred.

The results of the morphological examination were similar to those of the  $^{51}\text{Cr}$  releasing assay, *i. e.*, % phagocytosis was low at 1 h (0.4%), while % binding was significantly high at 1 h (31.0%), although % binding decreased markedly after 6 h of incubation (Table 1).

*The cytotoxicity of armed peritoneal exudate cells against Tg-coated chicken erythrocytes.* Cytotoxicity developed slowly and significantly. On the other hand, net % phagocytosis increased rapidly and greatly, reaching a plateau within 2 h ( $46.9 \pm 0.7\%$ ).

*The blocking effect of non-specific immunoglobulin-G.* As shown in Fig. 6, net %  $^{51}\text{Cr}$  release was suppressed even by 1 : 200 diluted normal human serum (NHS) or 10  $\mu\text{g}/\text{ml}$  of Cohn's fraction II (Fr II).

## Discussion

Antibody-dependent cell-mediated cytotoxicity (ADCC) has been described in autoimmune thyroiditis (26-31), and cytotoxicity mediated by patients' lymphoid cells has been reported in Hashimoto's thyroiditis (29, 30, 32). Instead of thyroid epithelial cells, Tg-coated chicken erythrocytes have also been used as target cells. It has been demonstrated that normal lymphoid cells significantly destroyed target cells which were preincubated with antisera or incubated simultaneously with antisera. Some authors have reported that the degree of cytotoxicity correlates with the ATgA titer. Wasserman *et al.* (28) reported that hemagglutinating antibody (mainly IgM) and antibody which mediates ADCC (IgG) were different from each other, and Bogner *et al.* (31) reported that antimicrosomal antibodies

were responsible for the cytotoxic effect. None of these authors, however, discussed monocyte-mediated cytotoxicity. In the present study, the cytotoxicity of human monocytes against Tg-coated chicken erythrocytes in the presence of the sera of patients with Hashimoto's thyroiditis closely correlated with the logarithm of the ATgA titer. The low cytotoxicity of the sera of patients with Graves' disease may have resulted from the low ATgA titers of the sera tested.

Although there are few reports describing cytophilic antibodies in human diseases (33, 34), it is certain, according to Shishiba (33) and Suzuki *et al.* (23-25), that there are cytophilic antibodies against thyroglobulin or the microsomal fraction of the thyroid in the sera of some patients with Hashimoto's thyroiditis. As shown in the present study, this cytophilic antibody was cytophilic even for heterologous effector cells, *i. e.*, guinea-pig peritoneal exudate cells, as well as human monocytes, and mediated extracellular cytolysis and phagocytosis of the target cells.

When normal monocytes or peritoneal exudate cells were incubated with Tg-coated chicken erythrocytes in the presence of the sera of patients with Hashimoto's thyroiditis, net % phagocytosis, which increased greatly within 2 h, decreased gradually thereafter. This decrease may suggest the release of free isotope from effector cells after intracellular digestion of previously engulfed target cells.

The question should be resolved why  $^{51}\text{Cr}$ -release and phagocytosis occurred much more slowly and to a lesser extent when armed monocytes were effector cells against Tg-coated chicken erythrocytes than when non-armed monocytes were effector cells against ATgA-bound Tg-coated chicken erythrocytes. Indeed, no significant phagocytosis occurred in the former case. Morphological observations showed similar

results. The difference between cytotoxicity of armed and non-armed monocytes may be explained by the following possibilities: (i) There may be fewer ATgA arming monocytes than sensitizing target cells. Imir *et al.* (35) reported that arming of effector cells with cytophilic antibodies required a higher concentration of antibodies than that required for target cell sensitization in order to induce the same degree of cytotoxicity. Possible causes of the small amount of ATgA which arm monocytes may be; (a) cytophilic antibodies bound to effector cell membrane may include not only ATgA but also AMcA or other non-specific antibodies which compete for Fc-receptors on effector cells and block ATgA-mediated cytotoxicity. (b) The receptors for cytophilic antibodies may be scarce originally. In fact, armed guinea-pig peritoneal exudate cells, which are considered to have more Fc receptors than human monocytes (36), could engulf target cells. (c) Some cytophilic antibodies may be detached from the receptors during the washing procedure because of their weak affinity for the receptors. (d) Some ATgA arming monocytes may be internalized together with the receptors before reacting with target cells because of the thermal change from 4°C to 37°C. (ii) Antibodies arming effector cells and those sensitizing target cells, although the latter may include the former, may be different from each other and may attach to distinct Fc-receptors on monocytes and have different functions (37, 38). In our preliminary experiment, monocyte-arming ATgA extracted from the sera of patients with Hashimoto's thyroiditis using an affinity column did not mediate phagocytosis (data not shown). (iii) The movement of the monocyte membrane may be restrained by cytophilic antibody attachment to phagocytic receptors. The bound cytophilic antibodies may change the electric charge or conforma-

tion of the monocyte membrane and suppress the sequence of the "zipper mechanism" of phagocytosis proposed by Griffin *et al.* (39). This possibility, however, does not fit the marked phagocytosis shown by guinea-pig peritoneal exudate cells. (iv) Finally, it is likely that sensitized target cells become so fragile during the sensitization procedure that they are broken more easily than those not sensitized.

We examined the blocking effect of non-specific IgG on the cytotoxicity because the cytotoxicity was lower when effector cells and target cells were incubated simultaneously with antisera than when effector cells were incubated with sensitized target cells. The cytotoxicity was blocked by normal human sera or non-specific IgG, suggesting that normal sera and non-specific IgG protect thyroid epithelial cells *in vivo*.

We tried to elucidate the detailed mechanism of ADCC mediated by human monocytes and the significance of cytophilic ATgA using Tg-coated chicken erythrocytes as target cells. It may not be adequate to regard Tg-coated chicken erythrocytes as thyroid epithelial cells, but thyroid cell membrane bound Tg has been reported (40, 41). Tg-coated chicken erythrocytes are convenient to use and are available to the examination of the ADCC mechanism related to Hashimoto's thyroiditis. The present study showed that normal monocytes are potent effector cells in ADCC which may be an important destructive mechanism in Hashimoto's thyroiditis, and the possibility was demonstrated that cytophilic antibodies present in the sera of patients with Hashimoto's thyroiditis render non-immune peripheral monocytes cytotoxic against cells bearing thyroid antigens *in vivo*.

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