Clin Exp Immunol 1994; 95:115–121

Immune complex-degradation ability of macrophages in MRL/Mp-*lpr*/*lpr* lupus mice and its regulation by cytokines

H. KANNO, O. TACHIWAKI, M. NOSE & M. KYOGOKU *Department of Pathology, Tohoku University School of Medicine, Sendai, Japan*

(Accepted for publication 15 September 1993)

SUMMARY

Impaired clearance of circulating and/or deposited immune complexes (IC) by the mononuclear phagocytic system is one of the major factors in the pathogenesis of IC diseases. MRL/Mp-*lpr*/*lpr* (MRL/*lpr*) lupus mice spontaneously develop a lethal glomerulonephritis associated with IC deposition. The ability of macrophages to degrade phagocytozed IC and regulation of this degradation in MRL/*lpr* mice were examined. In 4-month-old MRL/*lpr* mice, macrophages accumulated in the affected glomeruli and these macrophages contained many phagosomes containing electron-dense bodies. When culture supernatant of human T cell line HUT102 was administered intraperitoneally into disease-bearing MRL/*lpr* mice, degradation of these electron-dense bodies in the macrophages in glomeruli was noted. We developed a quantitative *in vitro* assay for IC degradation activity of MRL/*lpr* resident peritoneal macrophages (RPM) using peroxidase-labelled IC derived from MRL/*lpr* mouse sera. The ability of the RPM to degrade IC was remarkably enhanced by the pretreatment with HUT102 cell products and the related human recombinant cytokines, lymphotoxin and IL-1α. Moreover, pretreatment of RPM from non-diseased MRL/Mp-+/+ mice with the culture supernatant of spleen cells from diseased MRL/*lpr* mice reduced their IC degradation activity. These results suggested that the ability of macrophages to degrade IC in MRL/Mp strains of mice is under the regulation of cytokines, and the impaired ability in the disease-bearing mice may be the result of abnormalities in the cytokine system in these mice.

Keywords immune complex disease glomerulonephritis phagolysosome lymphotoxin IL-1

INTRODUCTION

In the pathogenesis of human immune complex diseases, such as systemic lupus erythematosus (SLE), clearance of soluble immune complexes (IC), particularly via Fc receptor-mediated phagocytosis, is impaired [1]. Impaired clearance of IC results in vascular and glomerular deposition of IC and IC-mediated tissue injury caused by subsequent complement activation, such as complement-mediated cytolysis and leucocyte-mediated tissue destruction. Although macrophages play a role primarily as scavengers of IC, macrophages can also work directly to cause lesions as effector cells in tissue injury through lysosomal hydrolases [2] and superoxide [3–5] release triggered by IC. However, there are few reports about the regulation of macrophage–IC interaction in IC diseases.

MRL/Mp-*lpr*/*lpr* (MRL/*lpr*) mice develop lupus-like nephritis associated with severe IC deposition by almost 4 months of age and have been well studied as a model for SLE [6,7]. In these mice, the lymphoproliferation of $CD4^-$, $CD8^-$, and $B220^+$ T lymphocytes (double negative T cells (DNT cells)) encoded by the *lpr* gene [8–11] is critical for the development of glomerulonephritis in the face of the genetic background of an MRL/Mp strain of mice [12,13], since MRL/Mp-+/+ (MRL/+) mice or other *lpr*-bearing mice such as C3H/HeJ-*lpr*/*lpr* and C57Bl/6-*lpr*/*lpr* never develop glomerulonephritis at least until 5 months old. However, the relationship between the proliferation of DNT cells and the onset of IC diseases in these mice is still unclear.

Macrophages may also play a role in these diseases. Alterations in several functions of macrophages in MRL/*lpr* mice compared with those in MRL/+ mice, such as Ia expression [14–18], antigen presentation [17–19], phagocytosis [18,20,21], Fc receptor expression [18,20], antibody-dependent cellular cytotoxicity (ADCC) [22], tumour cytotoxicity [14,21,22], lysosomal enzyme activity [21], and superoxide production [21,22], have been reported. These functional alterations of MRL/*lpr* macrophages may be crucial in the onset and development of IC diseases.

In this study we assess the degradation of ingested IC in macrophages of MRL/Mp strains of mice from the view of

H.K. present address: Department of Pathology, Osaka University Medical School, Suita, Osaka 565, Japan.

Correspondence: Masato Nose MD, Department of Pathology, Tohoku University School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980, Japan.

macrophage–IC interaction. MRL/*lpr* macrophages showed impaired degradation of IC *in vivo* and *in vitro*. Moreover, IC degradation ability in macrophages of MRL/Mp strains of mice may be under the control of cytokines.

MATERIALS AND METHODS

Mice

MRL/*lpr* and MRL/+ mice were purchased from the Jackson Laboratory (Bar Harbor, ME) in 1985 and have been bred in closed colonies and housed in the clean rooms of the Animal Research Institute of Tohoku University School of Medicine. MRL/*lpr* mice, 4–6 months old, and 4-month-old MRL/+ mice were used in this study. We confirmed that MRL/*lpr* mice, but not MRL/+, in our colonies developed glomerulonephritis at these ages.

Histopathology of glomerular lesions

Four-month-old MRL/*lpr* mice were killed under ether anaesthesia. Kidneys were removed, fixed with 10% formalin in 0·01 M phosphate-buffer pH 7·2, and embedded in paraffin. These paraffin sections were stained with haematoxylin and eosin (H&E) for histopathological examination under light microscopy. For immunofluorescence studies, kidneys were fixed with periodate-lysine-paraformaldehyde (PLP) solution [23], embedded in OCT compound (Miles Laboratories Inc., Elkhart, IN), frozen in a mixture of acetone and dry ice, and stored at −70°C until use. Frozen sections (3 μm) of kidneys were fixed with acetone at 4°C for 5 min. Immunostaining for IgG, IgM, or C3 was performed in a direct method using FITC-conjugated rabbit anti-mouse IgG, IgM, or C3 antibodies (Miles Laboratories Inc., Naperville, IL), respectively. Murine Mac-2 antigens [24] were detected by using rat anti-Mac-2 antibodies (Hybritech Inc., San Diego, CA), followed by biotinylated rabbit anti-rat IgG antibodies and FITC-labelled avidin (Vector Laboratories Inc., Burlingame, CA).

For electron microscopy, small pieces of renal cortices were fixed with 2·5% glutaraldehyde, and postfixed with 2% osmium tetroxide. After dehydration with ethanol, they were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead nitrate.

Human T cell line and supernatant preparation

Human T cell leukaemia virus type-I (HTLV-I) transformed human T cell line, HUT102 [25], was kindly donated by Dr H. Hemmi (Toho University School of Medicine, Tokyo, Japan) and grown in RPMI 1640 medium (Nissui, Tokyo, Japan) with 1% heat-inactivated fetal calf serum (FCS; Flow Laboratories, Irvine, UK). The supernatant was harvested by centrifugation (1500 ***g***, 10 min) and concentrated approximately 30-fold on Pellicon polysulfone membrane (Milipore Corp., Bedford, MA) with a molecular weight exclusion limit of 10 000. The concentrated material was dialysed against Eagle's minimum essential medium (MEM) (Nissui), sterilized with 0·22-μm Milipore filter (Milipore) and stored at −20°C until use.

In some experiments, the culture supernatant of HUT102 cells was prepared in Iscove's modified Eagle medium (GIBCO Laboratories, Grand Island, NY). Three millilitre per mice of the supernatant or the medium as the control were intraperitoneally injected into three 4-month-old MRL/*lpr* mice. Three days later, kidneys of these mice were removed and the glomerular lesions of these two groups were compared under electron microscopy.

Human cytokines

Recombinant human IL-1α (Ohtsuka Pharmaceutical, Tokyo, Japan), lymphotoxin (LT) (tumour necrosis factor-beta (TNF-β)) (Mitsubishi Chemical Co., Tokyo, Japan), granulocyte-macrophage colony-stimulating factor (GM-CSF; Genzyme, Boston, MA), and native human interferon-gamma (IFN-γ; Ohtsuka) were used for the pretreatment of mouse peritoneal macrophages *in vitro*.

Preparation of spleen cell culture supernatant

Spleens of mice were aseptically removed and minced with stainless steel mesh. Erythrocytes were lysed with hypotonic shock and spleen cells were resuspended at 1×10^7 cells/ml in Iscove's modified Eagle medium supplemented with 50 μg/ml of gentamicin sulphate (Sigma Chemical Co., St Louis, MO). These spleen cell suspensions were cultured at 37°C in 5% CO_2 for 48 h. The supernatant was harvested by centrifugation (1500 ***g***, 10 min), concentrated about 25-fold with 80% ammonium sulphate, and dialysed against MEM. After sterilization with 0·22 μm Milipore filter it was stored at −20°C until use.

*Preparation of horseradish peroxidase-labelled IC from MRL/*lpr *mice*

Five millilitres of pooled sera of female MRL/*lpr* mice (5–6 months old) were applied at room temperature onto Sephadex G-200 column (1·6 × 25 cm; Pharmacia, Uppsala, Sweden) equilibrated with Dulbecco's PBS (DPBS) containing 0·02% NaN_3. The excluded pool was collected as the fraction containing IC [26], since this fraction contained IgG as far as determined by ELISA on Nunc-Immunoplate (Nunc, Roskilde, Denmark) with horseradish peroxidase (HRP)-labelled rabbit anti-mouse IgG antibodies (Miles) [27]. This fraction was then dialysed against 10 mM carbonate buffer, pH 9·5, at room temperature overnight, labelled with HRP (Type VI; Sigma) [28], and finally adjusted to 5 ml (the same volume as the initial pooled sera) with Dulbecco's modified Eagle's medium (DMEM) (Nissui) supplemented with 5% heat-inactivated FCS to use as HRP-labelled IC from MRL/*lpr* mouse sera (MRL/*lpr*-IC).

Macrophages and time kinetics of IC degradation

Macrophages used in this study were obtained from resident peritoneal cells. In brief, resident peritoneal cells from a MRL/Mp strain of mouse were collected by peritoneal lavage with 5–10 ml of Hanks' balanced salt solution (HBSS) supplemented with 1 U/ml heparin. The collected cells were washed twice, resuspended in DMEM supplemented with 50 μg/ml gentamicin and 10% heat-inactivated FCS, and adjusted at the cell density of 5×10^5 macrophage-like cells/ml. Macrophage-like cells were morphologically defined by using Trück solution. Cell suspension (200 μl) was plated in a well of flat-bottomed, 96-well tissue culture plates (Nunc), and incubated for 4 h at 37°C in 5% CO_2 in air. After washing each well once with 200 μl of DMEM with 10% heat-inactivated FCS, still adherent cells were used as macrophages. Pooled peritoneal cells from 3–8 mice were used for each experiment, depending upon its scale.

In order to measure the time kinetics of IC degradation of macrophages, 50 μl of MRL/*lpr*-IC were added to macrophages

in each well. After incubation for the indicated periods at 37°C in 5% CO_2, each well was washed twice with 200 μl of the prewarmed DMEM containing 1% bovine serum albumin (BSA; Fraction V; Boehringer Mannheim-Yamanouchi, Tokyo, Japan). Then, 100 μl of the prewarmed DMEM with 10% heat-inactivated FCS were added to each well and the plates were incubated at 37°C for the indicated periods (digestion time). Each well was washed twice with cold HBSS containing 1% BSA and 0·02% NaN_3. Finally, 10 μl of 0·1% Triton X-100 in DPBS and 100 μl of reaction mixture (equal volumes of 0·03% H_2O_2 and 0·2 mg/ml 2,2′-azino-di-(3-ethylbenzthiazoline sulphonate (ABTS) in 100 mM citrate-phosphate buffer pH 5·3) were added to each well. These plates were left at room temperature for 20 min and the residual HRP activity was measured at OD_{405}–OD_{510}. Macrophage-associated HRP activity was represented after the subtraction of OD value of cell-free wells. Endogenous peroxidase activity of macrophages was undetectable in this system.

In order to confirm that the residual HRP activity in the macrophages reflected the amount of residual IgG derived from the phagocytozed IC, we also measured the amount of IgG in the same set of preparations. Instead of Triton X-100 and ABTS reaction mixture, 100 μl of distilled water was added to each well to lyse macrophages. The amount of IgG in the lysate was determined by ELISA on Nunc-TSP plate (Nunc) with alkaline phosphatase-labelled protein A (Boehringer Mannheim-Yamanouchi) as described previously [29]. The time kinetics of decrease in HRP activity of MRL/*lpr*-IC was correlated well with that in the amount of IgG (results not shown). Thus, the assay system described above was used for measuring the time kinetics of IC degradation of macrophages because of its fewer reaction steps and reproducibility.

Statistical analysis

Statistical significance was determined by Student's *t*-test.

RESULTS

*Microscopic findings in glomerular lesions in MRL/*lpr *mice*

Pathologic manifestations of glomerular lesions in MRL/*lpr* mice were characterized by diffuse cell-proliferative glomerulonephritis (Fig. 1a). IgG, IgM and C3 deposits were observed along glomerular capillary walls in a granular pattern and sometimes in a micronodular form (Fig. 1b). These glomerular lesions were characterized also by the accumulation of Mac-2-positive cells, indicating activated macrophages (Fig. 1c). Co-existence of both macrophages and IgG deposits suggested that macrophage–IC interaction may play a critical role in the development of glomerular lesions in MRL/*lpr* mice.

*Degradation of electron-dense deposits in MRL/*lpr *macrophages and the effects of cytokines* in vivo

Electron microscopic studies revealed that macrophages in glomeruli contained numerous electron-dense bodies in their phagosomes, not associated with phagolysosomal changes (Fig. 2a). These deposits seemed to be derived from phagocytozed osmiophilic materials deposited in glomerular subendothelial regions and mesangium (Fig. 2b). However, when glomeruli of the MRL/*lpr* mice treated with the culture supernatant of HUT102 cells 3 days earlier were examined (see Materials and Methods), such phagosomes with electron-dense deposits were

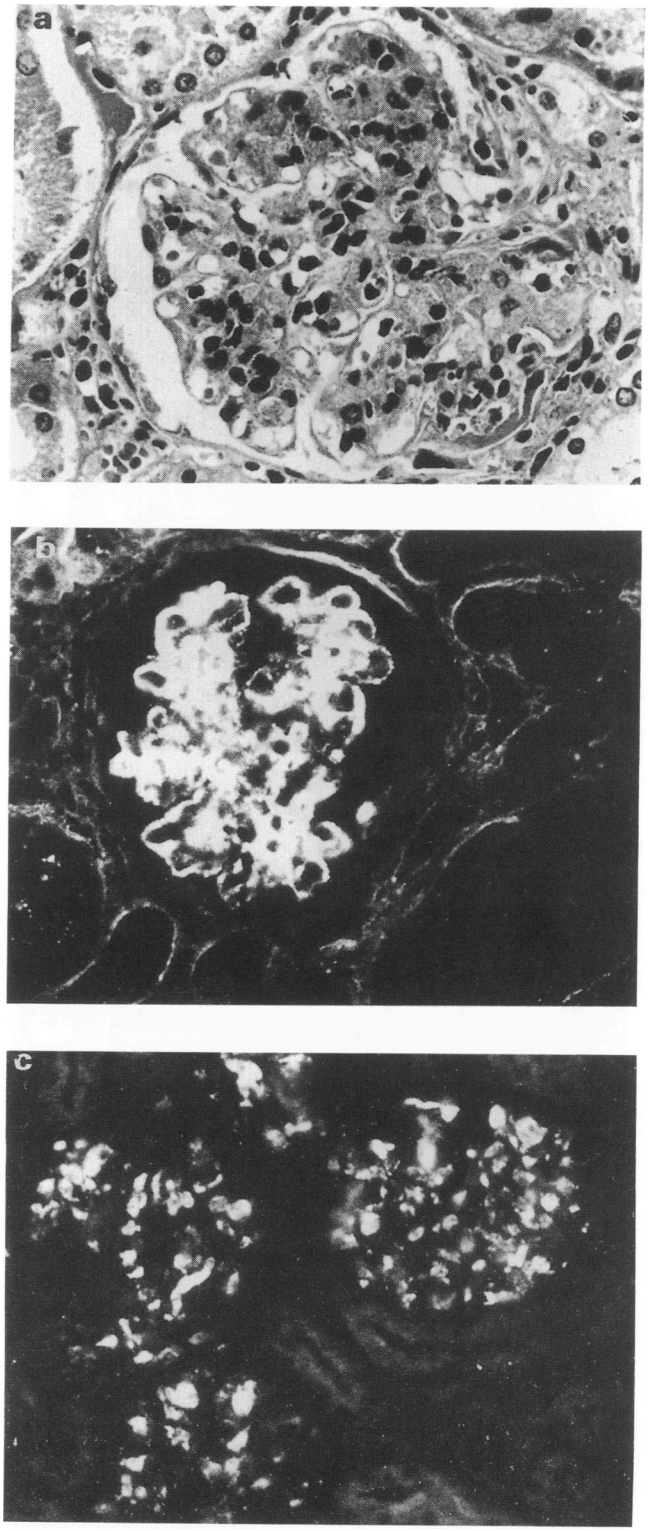

Fig. 1. Representative histopathological and immunohistochemical manifestations of glomerulonephritis in female 4-month-old MRL/*lpr* mice. (a) Diffuse cell-proliferative glomerulonephritis showing the accumulation of inflammatory cells and significant lobulation of glomerular tufts (haematoxylin and eosin stain, × 500). (b) IgG deposits along the capillary walls in a coarse granular pattern, partially in a micronodular form (immunofluorescence, × 375). (c) A large number of Mac-2-positive macrophages accumulate in an affected glomerulus (immunofluorescence, × 375).

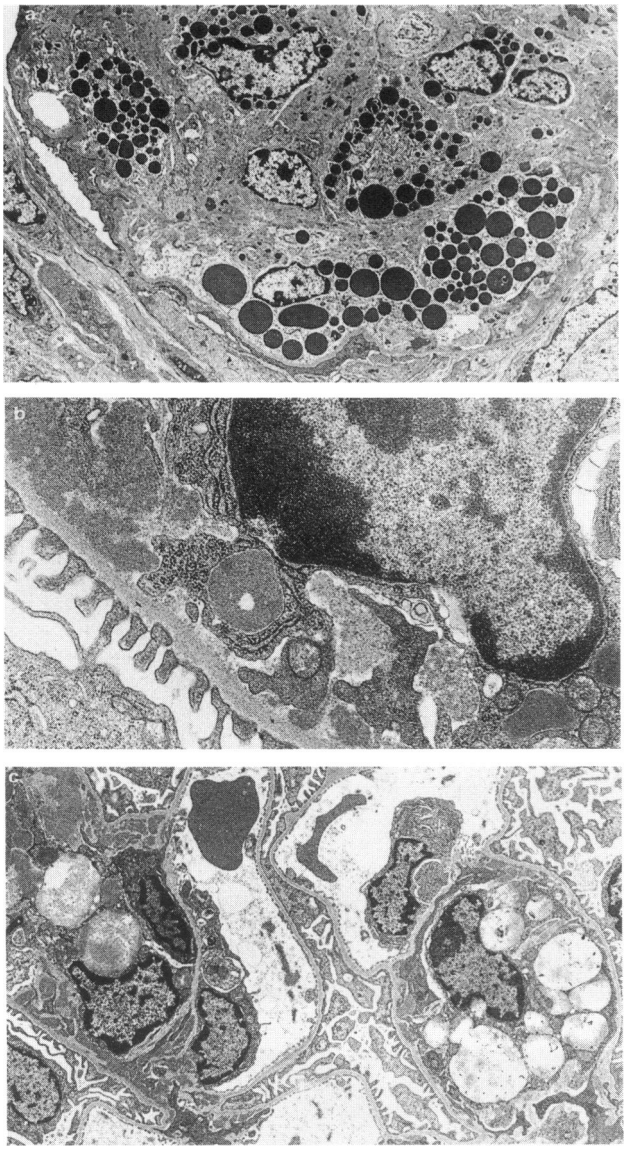

Fig. 2. Representative electron micrographs of the glomerular lesions of female 4-month-old MRL/*lpr* mice injected (c) or non-injected (a, b) with HUT102 CM (see Materials and Methods). (a) There are a lot of phagocytic cells with many osmiophilic materials, namely electron-dense bodies. Phagolysosomal changes are not observed (×3000). (b) Active phagocytosis of macrophage against osmiophilic subendothelial deposits (×9800). (c) Significant phagolysosomal changes of electron-dense bodies in macrophages, suggesting digestion of phagocytozed materials (×3800).

reduced in all of the treated mice, compared with the controls. Instead, we observed many vacuoles in the cytoplasm of these macrophages, indicating phagolysosomal changes of electron-dense bodies (Fig. 2c). Such vacuoles were not observed in the control mice.

*Quantitative analysis of IC degradation in MRL/*lpr *macrophages and the effects of cytokines* in vitro

The immunohistochemical and electron microscopic studies described above suggested that the ability of MRL/*lpr* macrophages to degrade phagocytozed IC deposited in glomeruli was

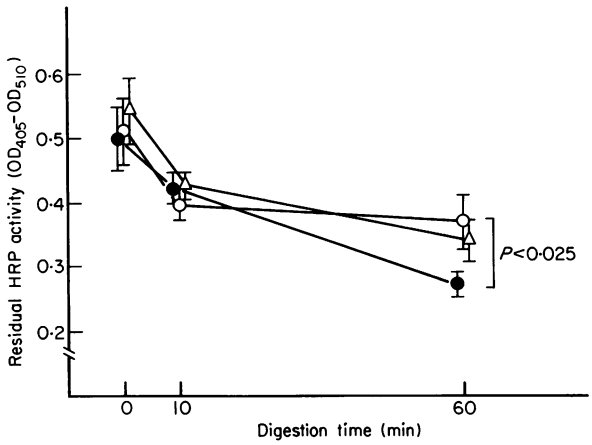

Fig. 3. Effects of HUT102 products on MRL/*lpr* macrophages in the degradation of MRL/*lpr*-IC. Resident peritoneal cells from MRL/*lpr* mice (4 months old) were plated in a well of the flat-bottomed, 96-well tissue culture plates (see Materials and Methods). Adherent cells were further incubated for 2 days in the absence (○) or presence of the concentrated HUT102 CM, diluted ×10 (●), or ×40 (△). After 2 h incubation with horseradish peroxidase (HRP)-labelled MRL/*lpr*-IC, HRP activity in macrophages was followed at indicated time points and represented as OD_{405}–OD_{510}. Results were represented as mean ± s.d. of quadruplicate wells. Very similar results in degradation time kinetics were obtained in experiments repeated twice.

augmented by HUT102 cell products. We set out to analyse the degradation of IC in macrophages *in vitro* using MRL/*lpr*-IC (see Materials and Methods).

In order to establish the assay system, we first incubated MRL/*lpr* macrophages with IC for varying times of 1–8 h, washed out excess IC, and then measured the kinetics of degradation of IC in macrophages. After 2 h of incubation with MRL/*lpr*-IC, degradation of IC in MRL/*lpr* macrophages was delayed compared with 1 h of taking up IC, but was not so different from 4 or 8 h of taking up (results not shown). Therefore, macrophages should be saturated or equilibrated with IC after the 2 h incubation. Thus, we incubated macrophages with MRL/*lpr*-IC for 2 h in the following experiments. In this condition, we measured the IC degradation activity of the macrophages at a saturated or equilibrated status with IC. This condition may reflect the *in vivo* situation of these macrophages, because a large amount of circulating IC exists in the sera of these mice [7,8].

We then examined the effects of HUT102 cell products on degradation of IC in MRL/*lpr* macrophages in this system. The cocultivation of MRL/*lpr* macrophages with the conditioned medium of HUT102 cells (HUT102 CM) after intake of MRL/*lpr*-IC showed no change in the kinetics of degradation of IC (results not shown). However, the pretreatment of MRL/*lpr* macrophages with HUT102 CM for 2 days enhanced the degradation of MRL/*lpr*-IC (Fig. 3). The enhancing effect of the culture supernatant of HUT102 cells on the degradation of IC was significant at 60 min after intake of IC ($P<0.025$). Thus, HUT102 CM contained certain soluble factors which enhanced the degradation of IC.

We next examined the effects of the pretreatment with several human cytokines on the degradation of MRL/*lpr*-IC in MRL/*lpr* macrophages. The cytokines examined here, IL-1α, LT (TNF-β), GM-CSF and IFN-γ, are known to be constitu-

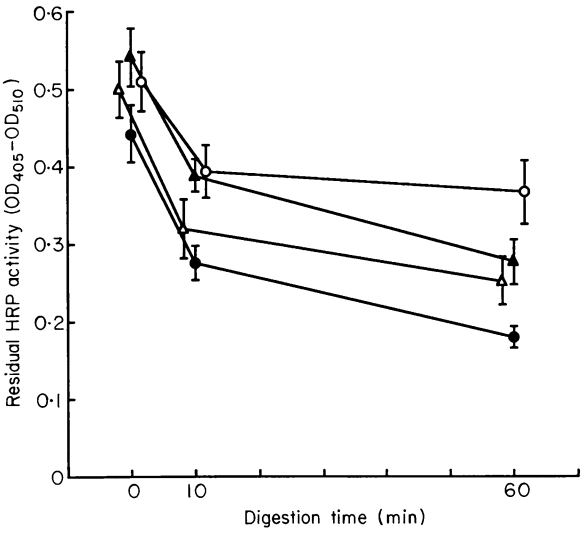

Fig. 4. Effects of recombinant human lymphotoxin (LT) on MRL/*lpr* macrophages in the degradation of MRL/*lpr*-IC. Peritoneal cell culture was prepared as described in Fig. 3. Adherent cells were incubated for 2 days in the absence or presence of LT. After 2 h incubation with horseradish peroxidase (HRP)-labelled MRL/*lpr*-IC, HRP activities in macrophages were followed at indicated time points and represented as OD_{405}–OD_{510}. Results were represented as mean ± s.d. of quadruplicate wells. ●, 500 U/ml; △, 50 U/ml; ▲, 5 U/ml; ○, none. This experiment was repeated three times with similar results in degradation time kinetics.

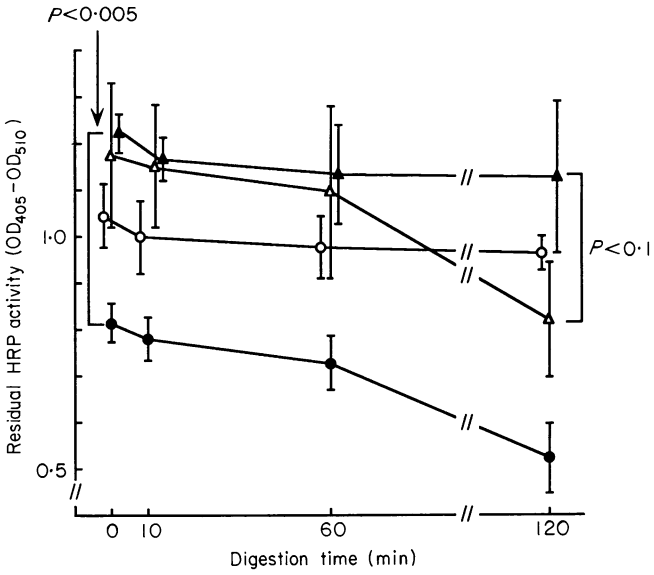

Fig. 5. Kinetics of the degradation of MRL/*lpr*-IC in MRL/*lpr* or MRL/+ macrophages and the effects of MRL/*lpr* spleen cell culture supernatant on MRL/+ macrophages on the degradation of MRL/*lpr*-IC. Resident peritoneal cells from MRL/+ mice (4 months old) were plated in a well of the flat-bottomed, 96-well tissue culture plates (see Materials and Methods). Adherent cells were further incubated for 2 days in the absence (●) or presence of the spleen cell culture supernatant of MRL/*lpr* mice (5–6 months old), × 10 diluted (▲) or × 40 diluted (△). Resident peritoneal cells from MRL/*lpr* mice (4 months old) were also plated in the same manner and adherent cells were incubated for 2 days without spleen cell culture supernatant (○). After 2 h incubation with horseradish peroxidase (HRP)-labelled MRL/*lpr*-IC, HRP activities in macrophages were followed at indicated time points and represented as OD_{405}–OD_{510}. Results were represented as mean ± s.d. of quadruplicate wells. This experiment was repeated three times with similar results in degradation time kinetics.

tively produced from HUT102 cells [30–33]. The pretreatment with LT seemed to enhance IC degradation in MRL/*lpr* macrophages in a dose-dependent manner (Fig. 4). Although the initial uptake of MRL/*lpr*-IC was almost the same between LT-treated and non-treated groups ($P > 0{\cdot}5$), the enhancing effect of LT (500 U/ml) was significant at 10 and 60 min ($P < 0{\cdot}005$ and $P < 0{\cdot}005$, respectively). IL-1α was also effective to a lesser extent (results not shown). On the other hand, GM-CSF and IFN-γ had no effect on the degradation of IC in MRL/*lpr* macrophages (results not shown).

These results indicate that IC degradation in MRL/*lpr* macrophages could be modulated by exogenously administered cytokines *in vivo* and *in vitro*.

Effects of MRL/lpr *cytokines on IC degradation*

The above results suggested that IC degradation activity of macrophages in non-manipulated and disease-bearing MRL/*lpr* mice might be impaired by cytokines produced constitutively in these mice. Thus, our last experiment was designed to examine the effects of cytokines produced by spleen cells of disease-bearing MRL/*lpr* mice on IC degradation in non-diseased MRL/+ macrophages.

Macrophages from 4-month-old MRL/+ mice were preincubated for 2 days with the indicated concentration of MRL/*lpr* (5–6-month-old) spleen cell culture supernatant (see Materials and Methods). The initial amount of MRL/*lpr*-IC retained in MRL/+ macrophages was increased ($P < 0{\cdot}005$, Fig. 5). Although the dose dependency of the MRL/*lpr* spleen cell culture supernatant on a delay in IC degradation in MRL/+ macrophages was not significant at 120 min ($P < 0{\cdot}1$), the delay in the higher concentration seemed to be compatible with that in non-treated MRL/*lpr* macrophages.

DISCUSSION

Evaluation of macrophage functions in MRL/*lpr* mice has yielded controversial results. Antigen presentation of MRL/*lpr* macrophages has been reported to be both normal [19] and decreased [17,18], and the phagocytic activity of MRL/*lpr* macrophages for sheep erythrocytes or opsonized sheep erythrocytes has been reported to be both enhanced [21] and decreased [18,20]. However, from a pathological point of view, MRL/*lpr* macrophages exhibit characteristics associated with tissue injury since ADCC activity [22], H_2O_2 release stimulated with phorbol myristate acetate [22], activity of lysosomal enzymes [21], and release of superoxide stimulated with opsonized zymosan [21] are enhanced in MRL/*lpr* macrophages. Moreover, clearance studies show that IC clearance in MRL/*lpr* mice *in vivo* is clearly delayed [34,35].

We found previously that phagocytic activity and lysosomal enzyme activity of macrophages in MRL/Mp strains of mice are controlled by the cytokines produced by MRL/*lpr* spleen cells [21]. Ia expression in MRL/*lpr* macrophages is also induced by MRL/*lpr* spleen cell products [14]. Although MRL/*lpr* macrophages derived from bone marrow cultures have been reported to exhibit some of the same functional alterations as those of macrophages from the disease-bearing mice [18], the terminal activation and/or functional modification of MRL/*lpr* macro-

phages responsible for the onset of disease seem to be regulated by the production of specific cytokines in these mice.

Therefore, we decided to study IC degradation ability of macrophages in MRL/*lpr* mice and its regulation by cytokines. In the present study, we demonstrated: (i) electron-dense body formation in macrophages in glomerular lesions of MRL/*lpr* mice; (ii) disappearance of these electron-dense bodies after administration of human T cell line-derived cytokines *in vivo*; (iii) enhancement of IC degradation activity in MRL/*lpr* macrophages by pretreatment with human T cell line-derived cytokines, or with human recombinant LT or IL-1α *in vitro*; and (iv) induction of impaired degradation of MRL/*lpr*-IC in MRL/+ macrophages by pretreatment with the spleen cell culture supernatant of disease-bearing MRL/*lpr* mice. These results indicate that cytokines regulate IC degradation in macrophages of MRL/Mp strains of mice, and that cytokines produced in MRL/*lpr* mice cause the impaired IC degradation in these mice, although our study did not clarify the critical cytokines affecting it.

Recently, a T cell cytokine, Eta-1, has been reported to be expressed at a very high level in DNT cells in MRL/*lpr* mice [36]. This cytokine binds to mouse peritoneal macrophages and attracts macrophages to a site of Eta-1 injection *in vivo* [37]. Although it is still unclear what kind of functional alteration Eta-1 induces in these mice, Eta-1 may be one of the cytokines affecting macrophage–IC interaction. The elevation of M-CSF is also reported in MRL/*lpr* mouse sera, but not in MRL/+ mice or other mouse strains bearing the *lpr* gene [38]. M-CSF increases the phagocytic but not bactericidal activity in macrophages of normal strains of mice [39]. Thus, M-CSF may be another candidate to generate the impaired degradation of IC in MRL/*lpr* macrophages. These findings suggest that a selected activation and/or differentiation of MRL/*lpr* macrophages to cause the impaired degradation of IC may be generated by several cytokines, but not by a single cytokine, which are involved in a macrophage-related cytokine network.

The defect in MRL/*lpr* macrophages which causes the impaired degradation of IC is still unclear. However, since the lysosomal enzyme activity *per se* of MRL/*lpr* macrophages is not decreased [21], dysfunction in phagosome–lysosome fusion such as is found in Chédiak–Higashi syndrome [40] may be involved. In fact, delayed fusion between acridine orange-labelled lysosomes and phagosomes containing opsonized zymosan can be observed in MRL/*lpr* macrophages (H. Kanno *et al.*, unpublished observation). Effects of cytokines on the fusion process should be further studied.

In this study we showed that IC degradation ability in macrophages of MRL/Mp strains of mice was under the control of several cytokines. Macrophages in MRL/*lpr* mice may differentiate into cells with a limited repertoire, being less able to degrade phagocytozed IC. Our results support the idea that selective alteration of macrophage functions by regulatory cytokines might be useful for the control of immune complex diseases.

ACKNOWLEDGMENTS

This work was supported by grants from the Research Committees, Ministry of Welfare of Japan, and by the Scientific Research Funds of the Ministry of Education, Science and Culture of Japan. We thank Kasumi Kobayashi and Yumi Shimoyama for technical assistance and animal care, Yumi Kanno for preparing the manuscript, Robert Evans, Gary Hettrick and Akio Ohkura for preparing the figures, and Marshall E. Bloom for critically reviewing the manuscript.

REFERENCES

1 Frank MM, Hamburger MI, Lawley TJ, Kimberly RP, Plotz PH. Defective reticuloendothelial system Fc-receptor function in systemic lupus erythematosus. N Engl J Med 1979; **300**:518–23.

2 Morrison AD, Pruzanski W, Ranadive NS. Release of lysosomal enzymes from human polymorphonuclear leukocytes by soluble intermediate immune complexes. Scand J Rheumatol 1978; **7**:241–6.

3 Connell PA, Seehra MS, Leslie RGQ, Reeves WG. Chemiluminescence of guinea pig peritoneal macrophages stimulated by immune precipitates and soluble immune complexes. Eur J Immunol 1980; **10**:966–8.

4 Baxter MA, Leslie RGQ, Reeves WG. The stimulation of superoxide anion production in guinea-pig peritoneal macrophages and neutrophils by phorbol myristate, opsonized zymosan and IgG2-containing soluble immune complexes. Immunology 1983; **48**:657–65.

5 Ward PA, Duque RE, Sulavik MC, Johnson KJ. *In vitro* and *in vivo* stimulation of rat neutrophils and alveolar macrophages by immune complex. Am J Pathol 1983; **110**:297–309.

6 Murphy ED, Roths JB. Autoimmunity and lymphoproliferation: induction by mutant gene *lpr*, and acceleration by a male-associated factor in strain BXSB mice. In: Rose NR, Bigazzi PE, Warner NL, eds. Genetic control of autoimmune disease. New York: Elsevier North Holland, 1978:207–21.

7 Andrews BS, Eisenberg RA, Theofilopoulos AN *et al.* Spontaneous murine lupus-like syndromes. J Exp Med 1978; **148**:1198–215.

8 Morse III HC, Davidson WF, Yetter RA, Murphy ED, Roths JB, Coffman RL. Abnormalities induced by the mutant gene *lpr*: expansion of a unique lymphocyte subset. J Immunol 1982; **129**:2612–5.

9 Wofsy D, Hardy RR, Seaman WE. The proliferating cells in autoimmune MRL/*lpr* mice lack L3T4, an antigen on "Helper" T cells that is involved in the response to Class II major histocompatibility antigens. J Immunol 1984; **132**:2686–9.

10 Nemazee DA, Studer S, Steinmetz M, Dembic Z, Kiefer M. The lymphoproliferating cells of MRL-*lpr*/*lpr* mice are a polyclonal population that bear the T lymphocyte receptor for antigen. Eur J Immunol 1985; **15**:760–4.

11 Mountz JD, Huppi KE, Seldin MF, Mushinski JF, Steinberg AD. T cell receptor gene expression in autoimmune mice. J Immunol 1986; **137**:1029–36.

12 Izui S, Kelley VE, Masuda K, Yoshida H, Roths JB, Murphy ED. Induction of various autoantibodies by mutant gene *lpr* in several strains of mice. J Immunol 1984; **133**:227–33.

13 Nose M, Nishimura M, Kyogoku M. Analysis of granulomatous arteritis in MRL/Mp autoimmune disease mice bearing lymphoproliferative genes; the use of mouse genetics to dissociate the development of arteritis and glomerulonephritis. Am J Pathol 1989; **135**:271–80.

14 Lu CY, Unanue ER. Spontaneous T-cell lymphokine production and enhanced macrophage Ia expression and tumoricidal activity in MRL-*lpr* mice. Clin Immunol Immunopathol 1982; **25**:213–22.

15 Kelley VE, Roths JB. Increase in macrophage Ia expression in autoimmune mice: role of the *lpr* gene. J Immunol 1982; **129**:923–5.

16 Kofler R, Schreiber RD, Dixon FJ, Theofilopoulos AN. Macrophage I-A/I-E expression and macrophage-stimulating lymphokines in murine lupus. Cell Immunol 1984; **87**:92–100.

17 Yamashita U. Dysfunction of Ia-positive antigen-presenting cells in autoimmune mice. Cell Immunol 1986; **97**:257–66.

18 Nakanishi A, Ashiba T, Imai Y, Osawa T. Functional defects of culture-grown bone marrow-derived macrophages from autoimmune MRL/MpJ-*lpr*/*lpr* mice. Microbiol Immunol 1987; **31**:155–67.
19 Scott Jr CF, Tsurufuji M, Lu CY, Finberg R, Sy M-S. Comparison of antigen-specific T cell response in autoimmune MRL/Mp-*lpr*/*lpr* and MRL/Mp-+/+ mice. J Immunol 1984; **132**:633–9.
20 Russell PJ, Steinberg AD. Studies of peritoneal macrophage function in mice with systemic lupus erythematosus: depressed phagocytosis of opsonized sheep erythrocytes *in vitro*. Clin Immunol Immunopathol 1983; **27**:387–402.
21 Nose M, Tachiwaki O, Miyazawa M, Kanno H, Kyogoku M. Macrophage functions and their regulation in MRL/*l* lupus mice. In: Kyogoku M, Wigzell H, eds. New horizons in animal models for autoimmune disease. Tokyo: Academic Press, 1987:219–31.
22 Dang-Vu AP, Pisetsky DS, Weinberg JB. Functional alterations of macrophages in autoimmune MRL-*lpr*/*lpr* mice. J Immunol 1987; **138**:1757–61.
23 McLean IW, Nakane PK. Periodate-lysine-paraformaldehyde fixative a new fixative for immunoelectron microscopy. J Histochem Cytochem 1974; **22**:1077–83.
24 Ho M-K, Springer TA. Mac-2, a novel 32,000 M_r mouse macrophage subpopulation-specific antigen defined by monoclonal antibodies. J Immunol 1982; **128**:1221–8.
25 Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA 1980; **77**:7415–9.
26 Nose M, Wigzell H. Biological significance of carbohydrate chains on monoclonal antibodies. Proc Natl Acad Sci USA 1983; **80**:6632–6.
27 Takahashi S, Nose M, Sasaki J, Yamamoto T, Kyogoku M. IgG3 production in MRL/lpr mice is responsible for development of lupus nephritis. J Immunol 1991; **147**:515–9.
28 Nakane PK, Kawaoi A. Peroxidase-labeled antibody a new method of conjugation. J Histochem Cytochem 1974; **22**:1084–91.
29 Heyman B, Nose M, Weigle WO. Carbohydrate chains on IgG2b; a requirement for efficient feedback immunosuppression. J Immunol 1985; **134**:4018–23.
30 Yamashita U, Shirakawa F, Nakamura H. Production of interleukin 1 by adult T cell leukemia (ATL) cell lines. J Immunol 1987; **138**:3284–9.
31 Kobayashi M, Plunkett JM, Masunaka IK, Yamamoto RS. Granger GA. The human LT system XII. Purification and functional studies of LT and "TNF-like" LT forms from a continuous human T cell line. J Immunol 1986; **137**:1885–92.
32 Salahuddin SZ, Markham PD, Lindner SG *et al.* Lymphokine production by cultured human T cells transformed by human T-cell leukemia-lymphoma virus-I. Science 1984; **223**:703–7.
33 Le J, Prensky W, Henriksen D, Vilcek J. Synthesis of alpha and gamma interferons by a human cutaneous lymphoma with helper T-cell phenotype. Cell Immunol 1982; **72**:157–65.
34 Jones FS, Pisetsky DS, Kurlander RJ. Defects in mononuclear phagocytic system (MPS) function in autoimmune MRL-*lpr*/*lpr* mice. Clin Immunol Immunopathol 1985; **36**:30–9.
35 Magilavy DB, Hundley TR, Steinberg AD, Katona IM. Hepatic reticuloendothelial system activation in autoimmune mice: differences between (NZB × NZW)F_1 and MRL-*lpr*/*lpr* strains. Clin Immunol Immunopathol 1987; **42**:386–98.
36 Patarca R, Wei F-Y, Singh P, Morasso MI, Cantor H. Dysregulated expression of the T cell cytokine *Eta-1* in $CD4^-8^-$ lymphocytes during the development of murine autoimmune disease. J Exp Med 1990; **172**:1177–83.
37 Singh RP, Patarca R, Schwartz J, Singh P, Cantor H. Definition of a specific interaction between the early T lymphocyte activation 1 (Eta-1) protein and murine macrophages *in vitro* and its effect upon macrophages *in vivo*. J Exp Med 1990; **171**:1931–42.
38 Yui MA, Brissette WH, Brennan DC, Wuthrich RP, Rubin-Kelley VE. Increased macrophage colony-stimulating factor in neonatal and adult autoimmune MRL-*lpr* mice. Am J Pathol 1991; **139**:255–61.
39 Cheers C, Hill M, Haigh AM, Stanley ER. Stimulation of macrophage phagocytic but not bactericidal activity by colony-stimulating factor 1. Infect Immun 1989; **57**:1512–6.
40 Stossel TP, Root RK, Vaughan M. Phagocytosis in chronic granulomatous disease and the Chediak–Higashi syndrome. N Engl J Med 1972; **286**:120–3.