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An increase in basal glucocorticoid concentration with age induces suppressor macrophages with high-density FcγRII/III

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SUMMARY

Ageing is usually accompanied by a decline in immune and neuroendocrine functions. To elucidate the mechanisms underlying age-related immunosuppression, the functions and surface phenotypes of peritoneal cells in the monocyte/macrophage lineage from old mice were investigated. The role of glucocorticoids (GC) in the immunomodulation was also examined. Proliferative responses of spleen cells from control mice stimulated with concanavalin A (Con A) were significantly suppressed by adding peritoneal exudate cells from old mice. Flow cytometry analysis revealed that the proportion of MAC-1$^+$ cells with a high density of type II or type III receptor for the Fc portion of IgG (FcγRII/III^{bright} cells) was increased markedly in the peritoneal exudate cells from old mice. The prominent suppressor activity for Con A responses of control spleen cells was found in the FcγRII/III^{bright} cells, whereas MAC-1$^+$ cells with a low density of FcγRII/III (FcγRII/III^{dull} cells) did not suppress the Con A responses. On the other hand, both the basal corticosterone concentrations in serum and the mRNA expression for GC receptor in peritoneal exudate cells increased significantly in old mice. Furthermore, the proportion of FcγRII/III^{bright} cells in peritoneal exudate cells from old mice was normalized on administration of the GC antagonist RU 38 486 (mifepristone). These results suggest that the increase in basal corticosterone concentrations in old mice induces the generation of FcγRII/III^{bright} suppressor cells, possibly leading to the immune-suppressive state.

INTRODUCTION

A progressive decline in the function of the immune system is one of several physiological changes thought to occur during mammalian ageing. Increased vulnerability of the aged animals to certain infections and neoplastic diseases characterize immunosenescence.[1,2] The age-related changes, such as diminished responses to stimulating signals and the reduced ability to proliferate and expand, have clearly been evident in lymphocytes, mainly in T cells.[3,4] In contrast, it remains unclear whether age-related alterations in monocyte and macrophage functions affect immunosenescence because studies on their functions in aged subjects have yielded conflicting results. Some studies found that there was no difference between monocytes from young and old subjects in the ability to kill bacteria or to generate cytokines,[5,6] although others found that the ability of macrophages to present antigens and to produce cytokines declined with ageing.[7,8]

Ageing has been defined as a general decline in bodily function associated with a decrease in the ability to maintain homeostasis, i.e. a decrease in the ability to cope with stress.[9] Glucocorticoids (GC) are now recognized to be among the most central of hormones secreted in response to stress after activation of hypothalamic-pituitary-adrenal axis (HPA) and are considered to be of critical importance in the control of homeostasis.[10] By contrast, excessive exposure to high GC concentrations will cause numerous stress-related diseases.[11] HPA dysfunction is often observed during ageing. Thus, the changes in glucocorticoid regulation during ageing and the pathogenic consequences of such dysregulations have long been speculated.[12,13] One issue has been whether aged animals respond appropriately to stress, or whether basal, under-stress and post-stress concentrations of GC differ by age. Although numerous studies examined GC concentrations in various aged animals, there has been considerable confusion as to the effect of age on basal, non-stressed corticosterone concentrations.

On the other hand, GC were the first neuroendocrine mediators found to be consistently associated with

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Abbreviations: Con A, concanavalin A; FcγRII/III, type II or III receptor for the Fc portion of IgG; GC, glucocorticoids; HPA, hypothalamic-pituitary-adrenal axis; mAb, monoclonal antibody; PCR, polymerase chain reaction; TdR, thymidine.

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immunosuppression.[14–16] They directly modulate immunological functions through GC receptors expressed in cells of the immune system and are considered to be responsible for the immunosuppression caused by the bulk of stressors.[17–20] However, the relationship between the circulating GC and the immunosenescence is not fully understood. In the current study therefore we investigated the effects of ageing on functions and cell-surface phenotypes of murine peritoneal exudate cells. In addition, the role of GC in the age-related changes of cells in monocyte/macrophage lineage was also examined.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice were obtained from Japan SLC Inc. (Shizuoka, Japan). Experiments were performed using mice between 8 and 10 weeks of age (young mice) or between 22 and 24 months of age (old mice). Animals were housed in groups of five or six at 25° with a 12-hr light/dark cycle. Food and water were available ad libitum.

Cell preparation and lymphocyte proliferative assay

Peritoneal exudate cells were induced in the mice by an intraperitoneal (peritoneal exudate) injection of 1 ml of liquid paraffin (Wako Pure Chemical Industries Ltd, Osaka, Japan).[21] In some experiments, animals were injected three times with the GC antagonist RU 38486 (10 mg/mouse at a time; a gift from Roussel UCLAF, Romainville, France) at the same time as a liquid paraffin injection and at intervals of 2 days. These reagents were suspended in 200 µl of phosphate-buffered saline (PBS) and were injected intraperitoneally. Control mice received PBS only. The dose of RU 38486 was based on our previous experiments.[18] Five or 6 days following induction, the peritoneal exudate cells were harvested, washed with PBS and suspended in PBS or in tissue culture medium consisting of RPMI 1640 (Life Technologies, Gaithersburg, MD) supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine (Life Technologies).

Spleen cell suspensions were prepared by gently passing the homogenized organ through a nylon mesh (64 µm; Abe Chemical Co., Chiba, Japan). Red blood cells were lysed in 0·16 M Tris-buffered ammonium chloride (pH 7·2; Wako Pure Chemical Industries) at 4° and washed twice in RPMI 1640. Cell viability was assessed by the trypan blue dye exclusion test. Cells were then suspended in the tissue culture medium. To determine mitogen-induced spleen cell proliferation, cells (4×10^5/well) in the tissue culture medium were cultured in quadruplicate wells, at 37° in a 5% CO_2-humidified atmosphere for 3 days with optimal concentrations of concanavalin A (Con A; 2·5 µg/ml; Sigma Chemical Co., St. Louis, MO), and 0·5 µCi/well [^{3}H]thymidine ([^{3}H]TdR; New England Nuclear, Boston, MA) was added for the final 8 hr of culture. Counts per min (c.p.m.) were determined in a liquid scintillation counter (Aloka Co., Tokyo, Japan). Results are expressed as [^{3}H]TdR incorporation by cells in mitogen-stimulated wells.

Immunofluorescence staining and flow cytometry

Flow cytometric analysis was carried out as described previously[22] using a FACStar PLUS (Becton Dickinson, Mountain View, CA). Prior to the immunofluorescence test, the peritoneal exudate cells (1×10^6) were incubated with mouse immunoglobulin in PBS at 5° for 30 min in order to avoid non-specific binding to the receptor for the Fc portion of immunoglobulin G (IgG). Thereafter, peritoneal exudate cells were treated with monoclonal antibody (mAb; 2·4G2, rat IgG2b, American Type Culture Collection, Rockville, MD) to the type II or type III receptor for the Fc portion of IgG (anti-FcγRII/III) and with fluorescein isothiocyanate (FITC)-labelled goat antirat immunoglobulin antibody (Caltag Laboratories, South San Francisco, CA). After extensive washing, the cells were treated with phycoerythrin (PE)-conjugated anti-MAC-1 mAb or anti-F4/80 mAb (Caltag Laboratories).[23] Data were illustrated by CELL Quest software (Becton Dickinson Immunochemistry Systems).

Cell fractionation and cell-mixing experiments

In some experiments, cells were sorted on FACStar PLUS. MAC-1$^+$FcγRII^{dull} cells and MAC-1$^+$FcγRII^{bright} cells were prepared as double-positive cells from peritoneal exudate cells stained as described above. These cells were serially diluted and added to the culture in which 4×10^5 non-manipulated spleen cells from control mice were stimulated with Con A (2·5 µg/ml). Three days later, [^{3}H]TdR incorporation was determined as described above.

Measurement of serum corticosterone

Serum corticosterone concentrations were determined by radioimmunoassay (Amersham Life Sciences, Arlington Heights, IL). Serum was diluted 1 : 5 with borate buffer (0·02 M, pH 7·4) and incubated at 60° for 30 min to denature corticosterone-binding proteins before the assay.

Reverse transcriptase–polymerase chain reaction

Total cellular RNA was extracted by the guanidinium-isothiocyanate method from peritoneal exudate cells. Single-strand cDNA was synthesized from 1 µg RNA with reverse transcriptase (RT) and was used for the polymerase chain reaction (PCR). Primer sequences were as follows: (β-actin) 5′ primer TGGAATCCTGTGGCATCCATGAAC, 3′ primer TAAAACGCAGCTCAGTAACAGTCCG; and (GC receptor) 5′ primer GCATGGAGAATTATGACCAC, 3′ primer ATCAGATCAGGAGCAAAGCA. cDNAs were amplified by PCR as follows: 26 cycles of 1 min at 94°, 1·5 min at 55° and 1·5 min at 72° for β-actin or with 30 cycles for the GC receptor. The PCR products were separated by electrophoresis on a 4% acrylamide gel and visualized by ultraviolet illumination after being stained with ethidium bromide. The amplified fragments were confirmed to correspond to GC-receptor mRNA by sequencing analysis.

Statistical analysis

When two means were compared, the Student's *t*-test for unpaired samples was used. For more than two groups, the statistical significance of the data was assessed by analysis of variance (ANOVA). When significant differences were found, individual comparisons were made between groups using the *t*-statistic and adjusting the critical value according to the Bonferroni method. Differences were considered significant at $P < 0{\cdot}05$. Data in the text and figures are expressed as means ± SEM.

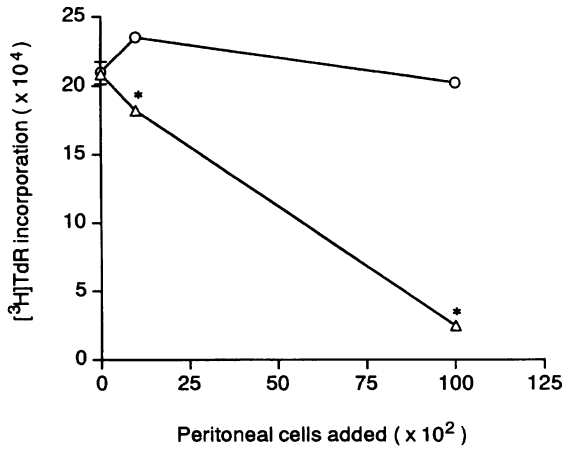

Figure 1. Effect of peritoneal exudate cells on Con A-induced proliferative responses. Various numbers of peritoneal exudate cells from young (○) or old (△) mice were added to 4×10^5 control spleen cells at the initiation of culture. Thereafter, the mixtures were cultured with Con A (2·5 μg/ml) at 37° for 72 hr. [^{3}H]TdR incorporation in the final 8 hr was determined for triplicate cultures. Representative results from two separate expriments are shown. Results are expressed as mean incorporation (c.p.m.)±SEM. Error bars are too small to be distinguishable in the figure. *Significantly lower than young mice at the same cell concentrations ($P<0·05$).

RESULTS AND DISCUSSION

The cells of the monocyte/macrophage lineage play a central role in the induction or suppression of lymphocyte activation or proliferation.[24,25] Such bidirectional regulations of macrophage activity result in tight control over an immune response. Several investigators have shown an age-related change in the number and activity of suppressor T cells, suggesting that decreased immune responses in animals may be caused by an imbalance between helper/inducer and suppressor cells [reviewed in reference 26]. The number and the activity of suppressor macrophages have been documented to increase in various infections or tumours.[27,28] However, little is known about the alterations in macrophage functions or populations with ageing. In the current study, we thus focused our investigation on the age-related changes of the cells of monocyte/macrophage lineage.

Changes in cell populations and functions of peritoneal exudate cells from old mice

Liquid paraffin-induced peritoneal exudate cells were collected from young and old mice and were analysed for their influences on immune responses. When serially diluted peritoneal exudate cells were added to the control spleen cell cultures stimulated with Con A, peritoneal exudate cells from old mice significantly suppressed the Con A responses in a dose-dependent manner, whereas the Con A responses were not inhibited, but rather enhanced, by peritoneal exudate cells from young mice (Fig. 1). These observations suggested that cells with suppressor function were generated in peritoneal exudate cells from old mice.

We then investigated the effects of ageing on the peritoneal exudate cell populations. Peritoneal exudate cells from young and old mice comprised similar proportions of MAC-1$^+$ cells (young, 77·3±1·9%; old, 71·5±3·2% ($P>0·10$)) or F4/80$^+$ cells (young, 66·7±3·3%; old, 67·2±7·8% ($P>0·10$)). Figure 2(a) shows the data comparing typical profiles of two-colour immunofluorescence staining with mAbs specific to

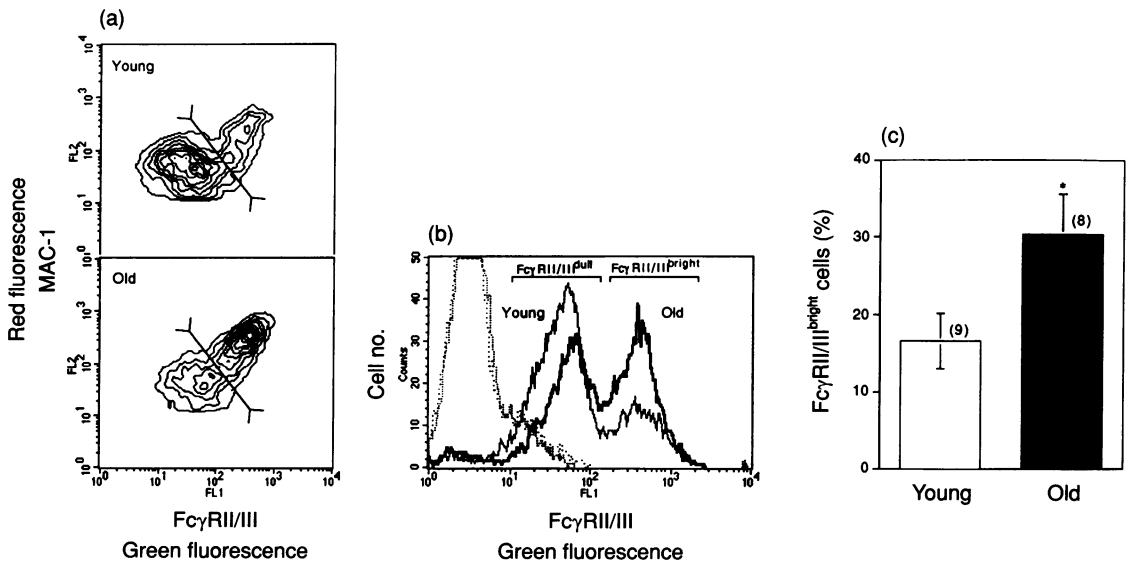

Figure 2. Effects of ageing on populations of peritoneal exudate cells. Expression of MAC-1 and FcγRII/III on peritoneal exudate cells was analysed by flow cytometry. The cells (1×10^6) were stained with anti-FcγRII/III mAb followed by FITC-anti-rat immunoglobulin, then reacted with PE-anti-MAC-1 mAb. Representative results from three separate experiments are shown. (a) Two-colour flow cytometric analysis of MAC-1 and FcγRII/III on peritoneal cells from young (upper panel) or old (lower panel) mice. (b) Single histogram of FcγRII/III on MAC-1$^+$ peritoneal cells from young (——) or old (——) mice. · · ·, ·····, negative control (cells stained with FITC-anti-rat immunoglobulin alone). (c) Each bar represents the mean percentage of FcγRII/III^{bright} cells in peritoneal exudate cells ±SEM The number of animals is indicated in parenthesis. *Significantly higher than young mice ($P<0·05$).

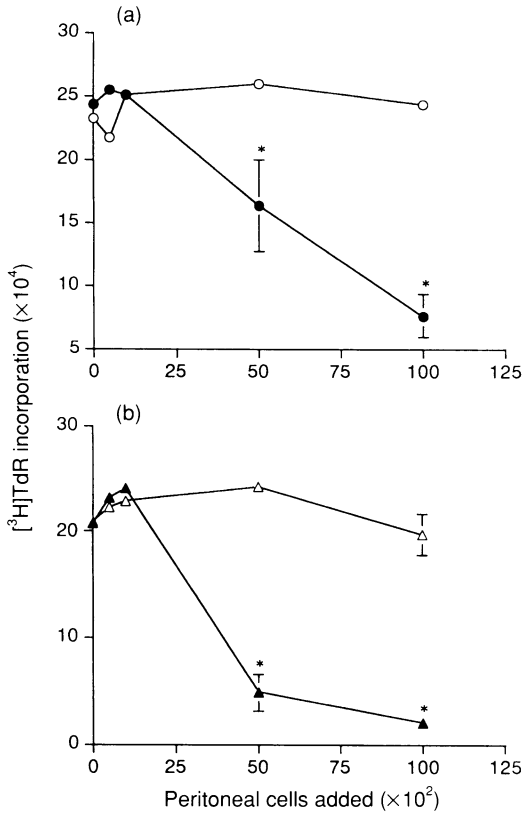

Figure 3. Effect of FcγRII/III$^+$ cells on Con A-induced proliferative responses. Various numbers of FcγRII/III^{dull} cells (○, △) or FcγRII/III^{bright} cells (●, ▲) from young (a) and old (b) mice were added to 4×10^5 control spleen cells at the initiation of culture. Thereafter, the mixtures were cultured with Con A (2·5 μg/ml) at 37° for 72 hr. [^{3}H]TdR incorporation in the final 8 hr was determined for triplicate cultures. Error bars are too small to be distinguishable in the figure. *Significantly lower than FcγRII/III^{dull} cells ($P<0·05$).

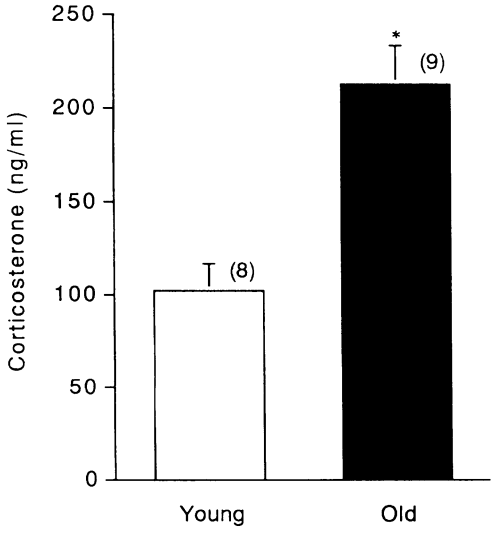

Figure 4. Effects of ageing on serum concentrations of corticosterone. The number of animals is indicated in parenthesis. *Significantly higher than young mice ($P<0·05$).

Figure 5. Expression of GC receptor mRNA on peritoneal exudate cells from young and old mice. Representative results from two separate experiments are shown. The expression of GC receptor and β-actin mRNA in peritoneal exudate cells from young and old mice were analysed by RT–PCR.

MAC-1 and FcγRII/III on peritoneal exudate cells from young and old mice. Two distinct cell populations (MAC-1$^+$FcγRII/III^{bright} cells and MAC-1$^+$FcγRII/III^{dull} cells) could be seen in both young and old groups. It is apparent from Fig. 2(b) and 2(c) that FcγRII/III^{bright} cells increased significantly among the peritoneal exudate cells from old mice. Greater than 95% of both FcγRII/III^{bright} cells from young and old mice were identified as mature macrophages using F4/80, a monoclonal antibody directed specifically against the mouse mature macrophages.

To elucidate the relationship between the suppressive function and the increased proportion of FcγRII/III^{bright} cells among the peritoneal exudate cells from old mice, FcγRII/III^{dull} and FcγRII/III^{bright} cells were purified by sorting in both young and old mice. These cells were then added to the Con A cultures of control spleen cells (Fig. 3). When serially diluted FcγRII/III^{bright} cells from young (Fig. 3(a)) or old (Fig. 3(b)) mice were added to 4×10^5 fresh spleen cells, the Con A responses were markedly suppressed in a dose-dependent manner. The Con A responses were almost completely inhibited at a dose of 1×10^4 cells per well. At the cell concentrations tested, FcγRII/III^{dull} cells from neither young nor old mice inhibited the Con A responses of control cells. It thus seems that the functional differences between peritoneal exudate cells from young and old mice were attributable to the age-related changes of cell populations in peritoneal exudate cells.

We have previously shown that acute cold stress increases the proportion of MAC-1$^+$FcγRII/III^{bright} suppressor macrophages in peritoneal exudate cells of mice.[18] In the current study, by contrast, peritoneal exudate cells were collected from young or old mice under normal conditions. Thus, the findings obtained here suggest that, even under such normal, unstressed conditions, there is a significant increase in the basal proportion of FcγRII/III^{bright} cells with suppressor function in old mice.

Participation of basal GC concentrations in the generation of FcγRII/III^{bright} cells from old mice

Our previous study has also revealed that the generation of MAC-1$^+$FcγRII/III^{bright} cells by acute cold stress is mediated by the action of GC through stimulation of the HPA.[18] Meanwhile, numerous studies have examined whether GC concentrations change with age but have failed to reach a consensus. Sapolsky[12] analysed in detail the data obtained from these studies on ageing and GC, and concluded that if

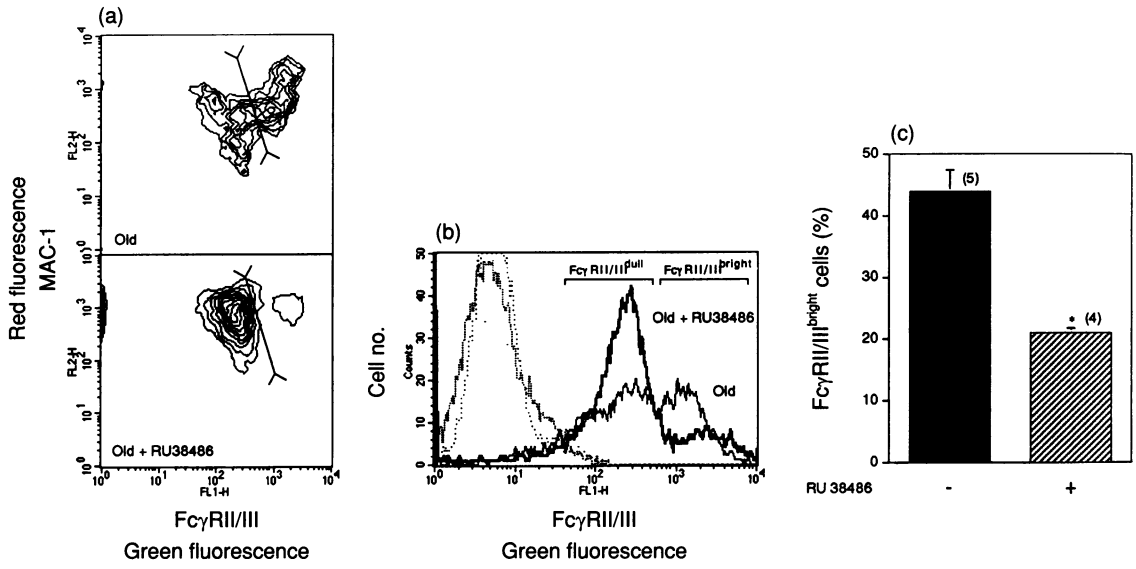

Figure 6. Effects of administration of RU 38486 on the generation of FcγRII/III^{bright} suppressor cells in old mice. Representative results from two separate experiments are shown. (a) Two-colour flow cytometric analysis of MAC-1 and FcγRII/III on peritoneal exudate cells from old mice (upper panel) or old mice treated with RU 38486 (lower panel). (b) Single histogram of FcγRII/III on MAC-1$^+$ peritoneal exudate cells from old mice (——) or old mice treated with RU 38486 (——). · · ·, ·····, negative control (cells stained with FITC-antirat immunoglobulin alone). (c) Each bar represents the mean percentage of FcγRII/III^{bright} cells in peritoneal exudate cells ± SEM. The number of animals is indicated in parenthesis. *Significantly lower than untreated old mice ($P < 0.05$).

rats are studied under truly basal, non-stressed circumstances, there is a marked increase in circulating corticosterone concentrations with age throughout the circadian cycle. In the current study, serum corticosterone concentrations in old mice were markedly higher than those in young mice (Fig. 4), supporting the conclusion by Sapolsky.[12] It is thus conceivable that the increase in basal GC concentrations affected the differentiation and function of the peritoneal exudate cells from old mice.

To examine the participation of the elevated concentrations of corticosterone in the changes of cell populations in peritoneal exudate cells of old mice, we attempted to detect mRNA for GC receptor and β-actin (as a control). Regardless of the almost uniform expression of mRNA for β-actin, old mice showed a high expression of GC receptor mRNA in peritoneal exudate cells compared with those from young mice (Fig. 5). Unfortunately, further analysis of GC receptor mRNA expression in terms of cell populations could not be carried out because we found a great deal of difficulty in achieving a sufficient number of cells by sorting. Next, the possible effect of endogenous GC on the generation of FcγRII/III^{bright} cells was analysed by administering the specific GC receptor antagonist (RU 38486). The treatment with RU 38486 normalized, i.e. rejuvenated the proportion of FcγRII/III^{bright} cells in peritoneal exudate cells from old mice (Fig. 6). These results suggest that the generation of MAC-1$^+$FcγRII/III^{bright} cells in old mice is mediated by the action of GC through the GC receptor.

In the current study, we demonstrated that MAC-1$^+$FcγRII^{bright} macrophages, which can exert prominent suppressor functions, increased in peritoneal exudate cells from old mice, and that the generation of the suppressor macrophages is mediated to a greater or lesser degree by the increase in basal GC concentrations, probably resulting from dysfunction of the HPA with ageing. It seems likely that an increase in suppressor macrophages with age may account for at least some of the declines in immune functions seen during ageing.

In our previous study, we have shown that acute cold stress induces suppressor peritoneal macrophages and that the suppressor macrophages strikingly suppress Con A responses of spleen cells from control mice by releasing nitric oxide.[19] In the present study, we observed that accumulation of nitrite in the lipopolysaccharide (LPS)-stimulated culture supernatants of peritoneal exudate cells from old mice were significantly higher than those from young mice (data not shown). Furthermore, hypersecretion of nitric oxide by macrophages obtained from aged mice following *in vitro* or *in vivo* stimulation with LPS was demonstrated.[8,29] Thus, the suppression of spleen cell Con A responses by MAC-1$^+$FcγRII^{bright} macrophages from aged mice might be attributable, in part, to nitric oxide. The critical mechanisms that regulate the cellular immune responses characterized in the current report, however, still remain to be elucidated. Further studies will be needed to clarify the underlying mechanisms by which neuroendocrine products modulate immune responses and alter disease susceptibility/severity in aged animals.

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REFERENCES

1. Lipschtz D.A., Golkdstein S., Reis R., Weksler M.E., Gressler R. & Weilan B.A. (1985) Cancer in the elderly: basic science and clinical aspect. *Ann Intern Med* **102,** 218.

2. Makinodan T., James S.J., Inamizu T. & Chang M.P. (1985) Immunologic basis for susceptibility to infection in the aged. *Gerontology* **30,** 279.
3. Thoman M.L. & Weigle W.O. (1989) The cellular and subcellular bases of immunosenescence. *Adv Immunol* **46,** 221.
4. Miller R.A. (1991) Aging and immune function. *Int Rev Cytol* **124,** 187.
5. Ligthart G.L., Schuit H.R.E. & Hijmans W. (1985) Subpopulations of mononuclear cells in ageing: Exporsion of the null cell compartment and decrease in the number of T and B cells in human blood. *Immunology* **55,** 15.
6. Nagel J.E., Chrest F.J. & Adler W.H. (1981) Enumeration of T lymphocyte subsets by monoclonal antibodies in young and aged humans. *J Immunol* **127,** 2086.
7. Higashimoto Y., Fukuchi Y., Shimada Y. *et al.* (1993) The effects of aging on the function of alveolar macrophages in mice. *Mech Ageing Dev* **69,** 207.
8. Chorinchath B.B., Kong L.-Y., Mao L. & McCallum R.E. (1996) Age-associated differences in TNF-α and nitric oxide production in endotoxic mice. *J Immunol* **156,** 1525.
9. Everitt A. & Meites J. (1989) Aging and antiaging effects of hormones. *J Gerontol* **44,** B139.
10. Berinardini R., Mauceri G., Iurata M., Chiarenza A., Lempereur L. & Scapagnini U. (1992) Response of the hypothalamic-pituitary-adrenal axis to interleukin 1 in the aging rat. *Prog Neuroendocrin Immunol* **5,** 166.
11. Munck A., Guyre P. & Holbrook N. (1984) Physiological functions of glucocorticoids during stress and their relation to pharmacological actions. *Endocrin Rev* **5,** 25.
12. Sapolsky R.M. (1992) Do glucocorticoid concentrations rise with age in the rat? *Neurobiol Aging* **13,** 171.
13. Stein-Behrens B.A. & Sapolsky R.M. (1992) Stress, glucocorticoids, and aging. *Aging Clin Exp Res* **4,** 197.
14. Blalock J.E. (1989) A molecular basis for bidirectional communication between the immune and Neuroendocrine systems. *Physiol Rev* **69,** 1.
15. De Souza E.B. (1993) Corticotropin-releasing factor and interleukin-1 receptors in the brain-endocrine-immune axis. *Ann NY Acad Sci* **697,** 9.
16. Jefferies W.M. (1991) Cortisol and immunity. *Med Hypoth* **34,** 198.
17. Lázár G., Lázár G. Jr., Husztik E., Duda E. & Agarwal M.K. (1993) The influence of antiglucocorticoids on stress and shock. *Ann NY Acad Sci* **761,** 276.
18. Kizaki T., Oh-Ishi S., Ookawara T., Yamamoto M., Izawa T. & Ohno H. (1996) Glucocorticoid-mediated generation of suppressor macrophages with high density FcγRII during acute cold stress. *Endocrinology* **137,** 4260.
19. Kizaki T., Oh-Ishi S. & Ohno H. (1996) Acute cold stress induces suppressor macrophages in mice. *J Appl Physiol* **81,** 393.
20. Kizaki T., Yamashita H., Oh-Ishi S., Day N.K., Good R.A. & Ohno H. (1995) Immunomodulation by cells of mononuclear phagocyte lineage in acute cold-stressed or cold-acclimatized mice. *Immunology* **86,** 456.
21. Kizaki T., Ishige M., Bingyan W. *et al.* (1993) Interleukin-1-dependent mitogenic responses induced by protoscoleces of *Echinococcus multilocularis* in murine lymphocytes. *J Leukoc Biol* **53,** 233.
22. Kizaki T., Kobayashi S., Ogasawara K., Day N.K., Good R.A. & Onoé K. (1993) Immune suppression induced by protoscoleces of *Echinococcus multilocularis* in mice.: Evidence for the presence of $CD8^{dull}$ suppressor cells in spleens of mice intraperitoneally infected with *E. multilocularis*. *J Immunol* **147,** 1659.
23. Austyn J.M. & Gordon S. (1981) F4/80, a monoclonal antibody directed specifically against the mouse macrophage. *Eur J Immunol* **11,** 805.
24. Unanue E.R. & Allen P.M. (1987) The basis for the immunoregulatory role of macrophages and other cells. *Science* **236,** 551.
25. Johnston R.B. Jr. (1988) Monocytes and macrophages. *N Engl J Med* **318,** 747.
26. Gottesman S.R.S., Thorbecke G.J. & Walford R.L. (1987) Specific and nonspecific suppressor cells in aged animals. In: *Aging and the Immune System* (ed. E. A. Goidl), p. 243. Marcel Dekker, New York.
27. Allison A.C. & Mansfield J.M. (1978) Mechanisms by which activated macrophages inhibit lymphocyte responses. *Immunol Rev* **40,** 1.
28. Metzger Z., Hoffeld J.T. & Oppenheim J.J. (1980) Macrophage-mediated suppression. I. Evidence fro participation of both hydrogen peroxide and prostagrandins in in suppression of murine lymphocyte proliferation. *J Immunol* **124,** 983.
29. Morrison D.C., Chen L.-C. & Pace J.L. (1993) Altered regulation of nitric oxide production *in vitro* by macrophages from senescent mice. *J Leukoc Biol* **54,** S75.