[Environmental Health and Preventive Medicine 6, 218–228, January 2002]

Review Article

Stress- and Aging-Associated Modulation of Macrophage Functions

Takako KIZAKI[1], Kenji SUZUKI[1], Tomomi OOKAWARA[2], Tetsuya IZAWA[3], Daizoh SAITOH[4], Shuji OH-ISHI[5], Keiichiro SUZUKI[2], Shukoh HAGA[6] and Hideki OHNO[1]

[1]*Department of Molecular Predictive Medicine and Sport Science, Kyorin University, School of Medicine, Tokyo*
[2]*Department of Biochemistry, Hyogo University of Medicine, Hyogo*
[3]*Department of Kinesiology, Graduate School of Science, Tokyo Metroporitan University, Tokyo*
[4]*Department of Traumatology and Critical Care Medicine, National Defense Medical College, Saitama*
[5]*5th Department of Internal Medicine, Tokyo Medical University, Tokyo*
[6]*Institute of Health and Sport Sciences, University of Tsukuba, Ibaraki*

Abstract

Effects of environmental (cold) stress and aging on cells in monocyte/macrophage lineage were investigated. We demonstrated that immune suppressive states seen in acute cold-stressed mice (8–10 weeks of age) is attributable to FcγRII^{bright} suppressor macrophages. Serum corticosterone levels were markedly increased in acute cold-stressed mice. In addition, expression of glucocorticoids (GC) receptor mRNA was observed in FcγRII^{bright} cells from these mice. The increase of FcγRII^{bright} cells in peritoneal exudate cells caused by acute cold stress was inhibited by adrenalectomy or administration of a saturating amount of the GC antagonist RU 38486 (mifepristone). On the contrary, administration of the GC agonist, dexamethasone, markedly increased the proportion of FcγRII^{bright} cells in peritoneal exudate cells of control mice. These results suggest that the generation of FcγRII^{bright} suppressor cells of monocyte/macrophage lineage by acute cold stress was mediated by action of GC through the GC receptor. We likewise found that the proportion of FcγRII^{bright} suppressor macrophages is increased in aged mice (22–24 months of age). Meanwhile, activated macrophages which function as antigen presenting cells were decreased in aged rats. Both the basal corticosterone concentrations in serum and the expression of mRNA for GC receptor in peritoneal macrophages increased significantly in aged animals, suggesting that these populational and functional changes of macrophages in aged animals were mediated, in part, by the increased basal levels of GC. This is probably being responsible for immunosenescence.

Key words: macrophage, stress, aging, glucocorticoid, immunosuppression

Introduction

Numerous investigations in both humans and animals have indicated that the immune system is influenced by stress[1,2]. Environmental temperature, one of the important stressors, modifies the immune capacity and influences both the frequency and the severity of infection[3,4]. Indeed, cold-exposed animals show qualitative and quantitative differences in the response to infections of various microorganisms[5–7]. However, the mechanisms underlying the altered immune responsiveness are not fully understood. Most previous studies have focused on analysis of lymphocyte functions. Thus far, the role of macrophages subsequent to a stressful stimulus has not been elucidated.

The cells of the monocyte/macrophage lineage play a central role in the induction and regulation of both innate and adaptive immune responses[8–9]. The innate immune response is initiated by recognition of molecular components of infectious agents. Binding of these foreign moieties to specific cellular receptors triggers distinct signaling pathways that lead to adaptive immune responses in order to eliminate the pathogens. The antigen processing and presentation and the production of soluble mediators by macrophages are essential requisites for the development of T cell-mediated immune responses[10]. In addition, macrophages are capable of expressing a large number of cell surface receptors and adhesion molecules, some of which are closely related to the capacity of the macrophage to achieve particular functions. That is to say, receptors for Fc portion of IgG (FcγR) are widely accepted to mediate mononuclear phagocyte functions such as endocytosis of immune complexes, Ab-dependent cell-mediated lysis[11–14], release of inflammatory mediators[15], and superoxide production[16]. Likewise, the type 3 complement receptor (MAC-1) plays a major role in cell-to-cell interactions to achieve immune

Received Sep. 17 2001/Accepted Nov. 16 2001
Reprint requests to: Takako KIZAKI
Department of Molecular Predictive Medicine and Sport Science, Kyorin University, School of Medicine, 6-20-2, Shinkawa, Mitaka, Tokyo 181-8611, Japan
TEL: +81(422)47-5511, FAX: +81(422)44-4427
E-mail: kizaki@kyorin-u.ac.jp

responses[17]. The expressions of these receptors and proteins are closely regulated and may be increased or decreased upon stimulation of the cells with a variety of inflammatory mediators and lymphokines. On the other hand, it has been reported that macrophages function as "natural suppressor" cells that down-regulate lymphocyte-dependent immune events both in vivo and in vitro. Recent studies have shown that activated macrophages appear to be more suppressive than their resident or non-activated counterparts[18,19]. The relationship between activated macrophages and suppressor macrophages, however, is not clear, because the macrophage population consists of heterogeneous cells in various states of activation or differentiation.

A major response to stress is the secretion of glucocorticoids (GC) following an activation of the hypothalamo-pituitary-adrenal axis (HPA) in mammalian species. GC were the first neuroendocrine mediators which were found to be consistently associated with immunosuppression[20–22], and directly modulate immunological functions through GC receptors expressed in cells of the immune system. Thus, it is considered that they are to a greater or lesser degree responsible for the immunosuppression caused by the bulk of stressors[23].

Meanwhile, a progressive decline in the function of the immune system is one of several physiological changes thought to occur during mammalian aging. Increased vulnerability of the aged animals to certain infections and neoplastic diseases characterizes immunosenescence[24,25]. 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, especially in T cells[26,27]. In contrast, it remains unclear whether age-related alterations in monocyte and macrophage functions affect immunosenescence. Aging 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[28]. Sapolsky[29] analyzed in detail the data obtained from numerous studies which examined the change of GC concentration in association with age, and concluded that there is a marked increase in the concentrations of circulating corticosterones with age throughout the circadian cycle.

Therefore, we investigated the effects of stress and aging on the functions and surface phenotypes of peritoneal cells in monocyte/macrophage lineage. We also examined the role of GC in the stress- and age-related changes of immune functions.

Immunoregulatory states in acute cold stressed or cold acclimated mice[30]

Responsiveness of the spleen cells from mice (8–10 week old) exposed to cold environmental temperature (5°C) (acute cold stress) to concanavalin A (Con A) was markedly suppressed, although the proportion of T cells in the spleen cells of acute cold stressed-mice increased significantly. When adherent cells from control mice were added to the T cell-enriched fraction prepared from acute cold-stressed mice, the Con A responses were restored to the normal level. In addition, suppression of Con A responses observed in the presence of excess adherent cells was considerably greater in the spleen cell cultures receiving adherent cells from acute cold-stressed mice than in those which received adherent cells from control mice. These findings suggest that the suppression seen in Con A responses of spleen cells from acute cold stressed-mice is not attributed to the malfunctions of the T cells but rather to the functional alteration of cells of mononuclear phagocyte lineage which play an important role in the Con A responses[8]. In our previous study, we have shown that macrophages which inhibit Con A responses by generating $CD8^+$ suppressor T cells are responsible for an immunosuppressive state observed in mice infected with Echinococcus multilocularis[31–34]. However, we could not detect suppressor T cells in cultures stimulated with Con A in the presence of adherent cells form acute cold-stressed mice. Thus, the depressed Con A responses of control spleen cells by adherent cells from acute-cold stressed mice were attributable to their direct suppressive effect on responding T cells.

In contrast to acute cold stress, when mice were exposed to cold environmental temperature for 3 weeks (cold acclimation), a significant increase of Con A responses and a decrease in the proportion of T cells were observed in the spleen cells. In the presence of adherent cells from control mice, however, T-enriched fraction from cold acclimated mice gave the same level of Con A responses as control T-enriched fraction did. Thus, it seemed unlikely that T cell functions of cold-acclimated mice were directly influenced by the stress. In addition, Con A responses of the T-enriched fraction from control mice were markedly enhanced by adding adherent cells from cold-acclimated mice as compared to those from control mice. These findings may permit us to conclude that the macrophages might play a major role in the enhancement of proliferative responses to Con A seen in spleen cells from cold acclimated mice.

When an organism undergoes a change of environmental temperature, the organism reacts to the stimulus and various responses occur during physiological adaptation or acclimation that may exert effects on the immune system. Thus, the alteration of cell-mediated immune functions and maintenance of a new immune balance may serve as an important index of cold acclimation.

Mechanisms underlying acute cold stress-induced immunosuppression[35]

We then focused our investigation on the regulatory function of cells of monocyte/macrophage lineage from acute cold-stressed mice. Liquid paraffin-induced peritoneal exudate cells were collected from these mice and were analyzed for their influences on cellular responses.

Peritoneal exudate cells from acute cold-stressed mice strikingly suppressed Con A responses of spleen cells from control mice. On the contrary, Con A responses were not inhibited but rather enhanced by peritoneal exudate cells from control mice. These observations suggest that cells with suppressor function were generated in peritoneal exudate cells of acute cold-stressed mice. Previously, Fujiwara et al.[36] have reported that surgical stress-induced immune suppression is due to antigen nonspecific suppressor T lymphocytes. However, we demonstrated that the suppression seen in Con A responses of spleen cells from acute cold-stressed mice was not attributable to the T cells. Rather, we showed a marked increase in the proportion of adherent cells with suppressor acitvity in peritoneal exudate cells of acute cold-stressed mice.

Macrophages have been shown to suppress lymphocyte proliferation by releasing hydrogen peroxide, prostaglandins, or other suppressive mediators[37]. Recently, Stuehr and Nathan[38] have demonstrated that activated macrophages produce a reactive radical nitric oxide (NO) during metabolism of L-arginine to NO_2^- or NO_3^-. The reactive nitrogen intermediates, in particular NO, exert anti-proliferative effects on a variety of cell types and can

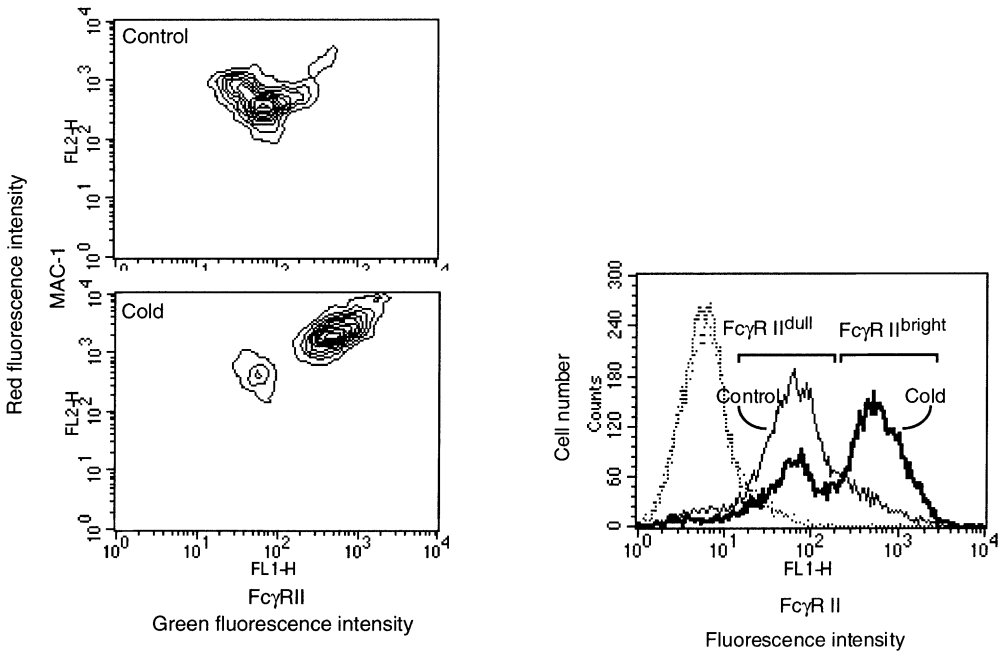

Fig. 1 Effects of acute cold stress on populations of peritoneal exudate cells. Both control and acute cold-stressed mice had received an injection of liquid paraffin alone. Expression of FcγRII, on peritoneal exudate cells was analyzed by flow cytometry. The cells (1×10^6) were stained with anti-FcγRII mAb followed by FITC-anti-rat immunoglobulin (Ig). Single histogram of FcγRII on peritoneal cells from control (——) or cold-stressed (------) mice. ····· , negative control (cells stained with FITC-anti-rat Ig alone). Reprinted from Kizaki et al.[40] by permission of *Endocrinology.*

well mediate the suppressive effects of macrophages on lymphocyte proliferation. We demonstrated that nitrite in abundant quantities was synthesized in spleen cell/adherent cell co-cultures in which T-lymphocyte proliferative responses to Con A were suppressed. Moreover, addition of NO synthase substrate analogue, NMMA, to the spleen cell/adherent cells co-cultures inhibited the nitrite synthesis and stopped the suppression of the T-lymphocyte proliferation. These results indicate that the suppressor activity of adherent cells is mediated, partially, by the NO derived from activated macrophages. Therefore, it seems likely that the NO-synthesizing pathway is, to a higher degree, up-regulated in adherent cells from acute cold-stressed mice compared with that from control mice.

Although increases of the number and the activity of suppressor macrophages have been documented to exist in cases of various infections or in tumors[39], little is known about the alteration of macrophage functions during responses to stress. The major new findings are that acute cold stress increases macrophages with augmented NOS II activity, which can exert prominent suppressor functions on T-lymphocyte proliferative responses, possibly leading to immunosuppressive states of acute cold-stressed animals.

Functions and surface phenotypes of murine peritoneal cells of monocyte/macrophage lineage from acute cold-stressed mice[40]

Peritoneal exudate cells were collected from control and acute cold-stressed mice and were analyzed for their influences on immune responses and surface characteristics. MAC-1^+FcγRII^{bright} cells were markedly increased in acute cold-stressed mice (Fig. 1)[40]. They strikingly suppressed Con A responses of spleen cells from control mice (Fig. 2)[40]. On the contrary, the Con A responses were not inhibited by MAC-1^+FcγRII^{dull} cells. The findings obtained suggest that the acute cold stress generates suppressor macrophages which may cause immune suppression. The FcγRII molecule is also a differentiation marker of cells of

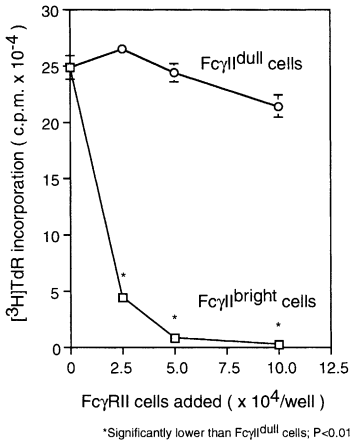

Fig. 2 Effect of FcγRII$^+$ cells on Con A induced proliferative responses. Acute cold-stressed mice had received an injection of liquid paraffin alone. Various numbers of FcγRII^{dull} cells (Cricles) or FcγRII^{bright} cells (Squares) from acute cold-stressed mice were added to 4×10^5 control spleen cells at the initiation of culture. Thereafter, the mixtures were cultured with Con A (2.5 μg/ml) at 37 C for 72 h. [^{3}H]TdR incorporation in the final 8 h was determined for triplicate cultures. Results are expressed as mean incorporation (cpm)±SE. * Significantly lower than control (P<0.05). Reprinted from Kizaki et al.[40] by permission of *Endocrinology.*

monocyte/macrophage lineage. It seems likely, therefore, that FcγRII^{bright} cells from acute cold-stressed mice are in a high state of differentiation. Indeed, the cells displayed an increased capacity for plastic adherence and had an increased phagocytic activity of IgG coated beads as described below. Further, it should be noted that the FcγRII^{bright} cells of the acute cold-stressed mice expressed considerably larger amounts of MAC-1 molecules on their surface (mean fluorescence intensity±SE: 1,943±67) than did those of control mice (880±109) (P<0.01). MAC-1 represents an adhesion molecule expressed on macrophages and plays a major role in

cell-to-cell interactions to achieve immune responses. In contrast, FcγRII^{dull} cells expressed smaller amounts of MAC-1 molecules than FcγRII^{bright} cells in both acute cold-stressed and control mice. The amount of MAC-1 molecules on the FcγRII^{dull} cells from acute cold-stressed mice was not significantly different from that on the cells from control mice (mean fluorescence intensity±SE: 288±88 (control); 316±123 (cold)), and these cells did not inhibit the Con A responses of control spleen cells. Both the functional analysis and the flow cytometric analysis of cell surface characteristics indicated that the FcγRII^{bright} cells in acute cold-stressed mice were at functionally high levels. It thus appears that peritoneal exudate cells are activated in some way following acute exposure to cold. The observations that activated macrophages, MAC-1^{+}FcγRII^{bright} cells, which are induced by acute cold stress, function as suppressor cells are congruent with the concept that activated macrophages are more suppressive than their resident or non-activated counterparts[19,41].

FcRs constitute a family of haematopoietic cell surface molecules capable of eliciting intracellular signals and triggering numerous effector responses upon crosslinking by their ligand, namely, the antibody-antigen complex. Type I and III FcRs for IgG (FcγRI, FcγRIII) are expressed primarily on cells of the myeloid lineage and they mediate effector functions (including phagocytosis, antibody-dependent cellular cytotoxicity and the release of inflammatory mediators), whereas type II receptors (FcγRII) are expressed on both myeloid and lymphoid lineages[42]. Despite its widespread distribution on both lymphoid and myeloid cells, the biological role of the low-affinity IgG receptor, FcγRII, is not fully understood. Defects in this receptor or in its signaling pathway in B cells result in perturbation in immune-complex-mediated feedback inhibition of antibody production[43–46]. Quite recently, elevated immunoglobulin levels in response to both thymus-dependent and thymus-independent antigens have been reported in FcγRII-deficient animals, suggesting that FcγRII acts as a general negative regulator of immune-complex-triggered activation[47]. It is considered, however, that FcγRII of macrophages is different from that of B cells. So far, the role of FcγRII of macrophages is not clarified. Further study is thus needed to understand the role of FcγRII molecules in the suppressor function of FcγRII^{bright} cells from acute cold-stressed mice.

There is a close relationship between the immune system and the neuroendocrine system in the body, both of which are involved in the maintenance of the body micro surroundings. Hypothalamic-pituitary-adrenal axis activation during physical or emotional stress leads to the secretion of GC from the adrenal gland and is considered to be the basis of stress-induced immunosuppression[22,48,49], but the mode of action of the complex interplay between hormones and the immune system is only partially understood.

We found that serum corticosterone levels were increased under acute cold stress. This observation is consistent with others[50,51]. Several studies have shown that GC regulate the differentiation of the cells of monocyte/macrophage lineage[52–54]. We also observed high expression of mRNA for GC receptor in peritoneal exudate cells from acute cold-stressed mice. It is conceivable that the increased GC levels during acute exposure to cold affect the differentiation and function of the FcγRII^{dull} cells, resulting in the generation of FcγRII^{bright} cells. Indeed, Girard MT et al.[53] showed that the expression of FcR mRNA was increased in human monocytes stimulated with GC for 2 h. To examine the possible effect of endogenous GC on the generation of FcγRII^{bright} cells under acute cold stress, we tested whether adrenalectomy (ADX)

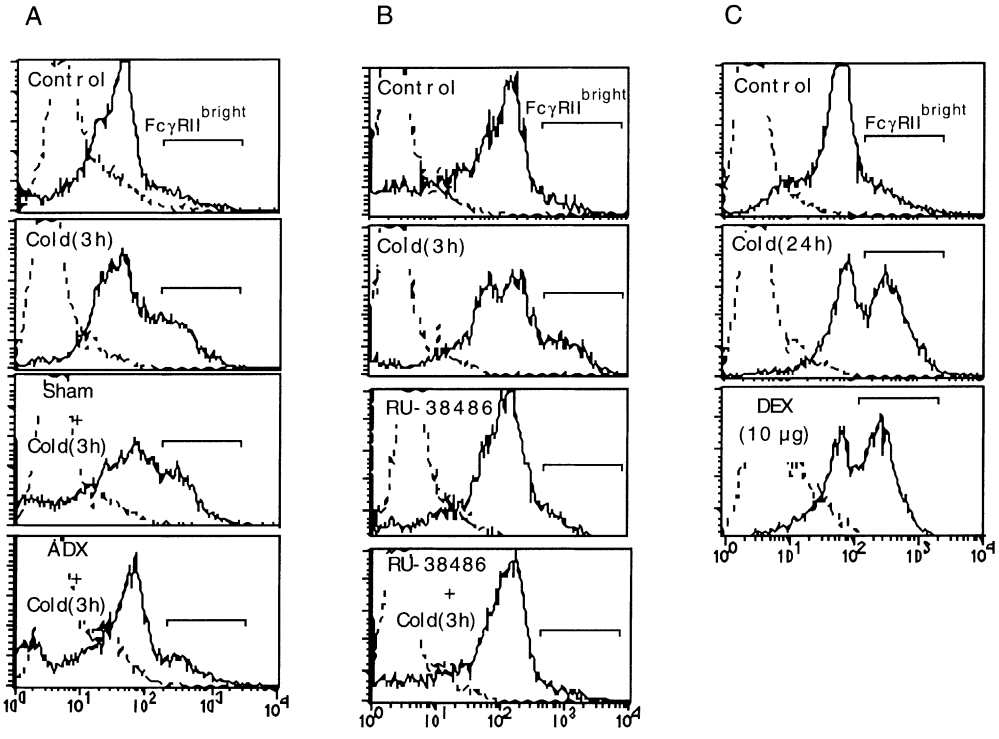

Fig. 3 (A) Effects of adrenalectomy on the generation of FcγRII^{bright} suppressor cells by acute cold stress. Representative single histograms of FcγRII on peritoneal exudate cells are shown. (B) Effects of administration of GC receptor antagonist, RU 38486, on the generation of FcγRII^{bright} suppressor cells by acute cold stress. Representative single histograms of FcγRII on peritoneal exudate cells are shown. (C) Effects of treatment of mice with GC agonist, DEX, on the proportion of FcγRII^{bright} suppressor cells. Representative single histograms of FcγRII on peritoneal exudate cells are shown. Reprinted from Kizaki et al.[40] by permission of *Endocrinology*.

or administration of a specific GC receptor antagonist (RU 38486) would inhibit the generation of FcγRII^{bright} cells. The proportion of FcγRII^{bright} cells was not increased in peritoneal exudate cells of ADX mice by 3-h cold stress, whereas it was significantly increased in sham-operated mice (Fig. 3A)[40]. Elimination of the effect of GC by treatment with RU 38486 also suppressed the generation of FcγRII^{bright} cells by 3-h cold stress (Fig. 3B)[40]. In addition, administration of GC alone remarkably increased the FcγRII^{bright} cells in peritoneal exudate cells of control mice (Fig. 3C)[40]. These results suggest that the generation of FcγRII^{bright} cells by acute cold stress is mediated by action of GC through the GC receptor.

Effect of swimming training on the generation of the MAC-1^{+}FcγRII^{bright} suppressor macrophages by acute cold stress[55]

In our previous study, the increase of FcγRII^{bright} suppressor macrophages in peritoneal exudate cells by acute cold stress appeared to be closely related to the decrease of rectal temperature, while the generation of the suppressor macrophages was inhibited in peritoneal exudate cells from cold-acclimated mice that had been exposed to cold for 3 weeks[56]. Therefore, we investigated whether improved cold tolerance by swimming training[57] would inhibit the immunomodulation by acute cold stress. As was anticipated, after a 6-wk endurance swimming training (5 times/wk), the proportion of FcγRII^{bright} cells in peritoneal exudate cells was unaffected by 3-h acute cold stress. Before acute cold exposure, corticosterone concentrations in serum from swimming-trained mice were similar to those from control mice. The 3-h acute cold stress did not significantly increase corticosterone concentrations in serum from swimming-trained mice. Therefore, it seems probable that the attenuated GC responses to acute cold stress in swimming-trained mice contribute to the lack of FcγRII^{bright} cell generation during acute cold stress, supporting the hypothesis that exercise benefits are mediated via acute stress reduction[58]. Cells of the monocyte/macrophage lineage play a central role in the induction or suppression of lymphocyte activation or proliferation[9]. Such bidirectional regulations of macrophage activity result in tight control over an immune response. Thus, an imbalance between helper/inducer and suppressor macrophages may influence immune responses[41]. Therefore, swimming training inhibited not only the decrease of body temperature but also the generation of suppressor macrophages during acute cold stress, probably improving resistance to illness such as infectious diseases in a cold environment.

Meanwhile, exercise may reduce the psychological and physical consequences of unavoidable or otherwise unmanageable stressor[59] perhaps by reducing anxiety[60], releasing endogenous opioid peptide[61], and/or decreasing autonomic reactivity[62]. Macrophages possess receptors to these hormones and neurochemicals, and their immune functions are affected positively or negatively by these mediators[63,64]. The role of these hormones in regulating macrophage function during exercise and stress is of obvious importance, however, has not been fully elucidated. We observed that acute cold stress markedly increased plasma norepinephrine levels in control mice, but not in swimming-trained mice. It is thus possible to assume that one or more hormone(s) or factor(s) mediates the swimming exercise-induced modulation of macrophage function.

The mechanisms for controlling host homeostasis[65]

Protein tyrosine kinases of *src*-family (*src*-PTK), encoded by c-*src*, c-*fgr*, c-*yes*, *fyn*, *lck*, *hck*, *lyn*, *blk*, and *yrk*, are expressed in various hematolymphoid cells in a lineage-specific manner and play essential roles in the intracellular transduction of signals initiated at the level of cell surface molecules that lack catalytic domains. The c-*fgr* protooncogene product, Fgr, is expressed in cells of monocyte/macrophage lineage and accumulating evidence suggests that tyrosine phophorylation events by *src*-PTK are crucial during the activation of macrophages upon stimulation with LPS[66,67]. Moreover, we have shown that a down-regulation of Fgr activity by overexpression of C-terminal Src kinase (Csk) decreases the production of NO and monokines[66,68]. We further found that upon stimulation with LPS, the activity of Fgr, the expression of IL-1β mRNA, and activity of IL-1 in the supernatants of adherent cells from acute cold-stressed mice were significantly lower than those in cells from control mice. In hematopoietic and immune system cells, failure to regulate signal transduction during cell activation can result in hyperresponsive states that lead to significant pathological sequelae, such as autoimmunity and excessive inflammation[69–71]. Medvedev et al.[72] have reported that prior exposure to LPS induces a transient state of cellular hyporesponsiveness to subsequent stimulation with LPS, known as endotoxin tolerance. This may be the mechanism constructed to protect the host from developing a shock syndrome caused by hyperactivation of monocytes and macrophages with bacteria and LPS. Thus, negative regulation of signal transduction cascades is necessary for homeostasis in a variety of systems. In the present work, negative regulation might occur in the adherent cells activated during acute cold stress, resulting in the tolerance to the subsequent stimulation probably by the similar mechanisms observed in LPS tolerant macrophages.

On the other hand, as already stated, acute cold stress generates FcγRII^{bright} macrophages which suppress T cell proliferative responses to Con A through NO[35,40]. It appears, therefore, that the increased adherent cells under acute cold stress might be dangerous cells rather than unwanted cells. One mechanism known to regulate potentially dangerous cells in the immune system is programmed cell death, namely, apoptosis. Accordingly, apoptosis would seem to be a logical candidate to participate in macrophage regulation. The number of adherent cells was markedly increased in peritoneal exudate cells during exposure to cold stress for 24 h and decreased thereafter. Interestingly, apoptotic cells were increased in peritoneal exudate cells from acute cold-stressed mice (Fig. 4)[65]. Furthermore, we found that the apoptotic cells resided not in the non-adherent cells but in the adherent cells from acute cold-stressed mice, thereby suggesting that the decrease in the adherent cell number observed in mice exposed to cold for 72 h was attributable to the activation-induced apoptosis of adherent cells. Although considerable advances have been made in elucidating the altered immune functions by stresses, the mechanisms involved in macrophage death are poorly understood, yet are crucial for understanding homeostasis in the host. These findings could provide evidence that apoptosis is an important homeostatic mechanism for controlling the number of activated macrophages.

Effects of aging on immune functions in mice[73]

Several investigators have shown an age-related change in the number and activity of suppressor T cells, indicating that decreased immune responses in animals may be due to an imbalance between helper/inducer and suppressor cells[74]. However, little is known about the alterations in macrophage functions or

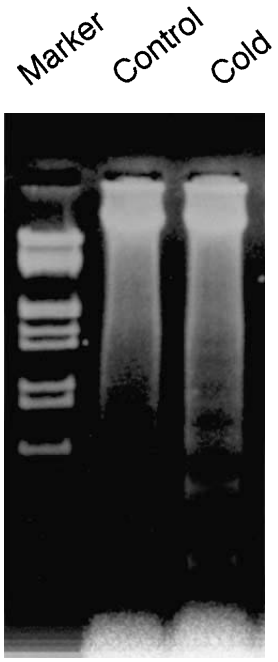

Fig. 4 Analysis of apoptosis in peritoneal exudate cells. Peritoneal exudate cells collected from five control or five acute cold-stressed mice were pooled respectively and used for the following analysis. DNA was extracted from peritoneal exudate cells of control and acute cold-stressed mice. DNA fragmentation was assessed by electrophoresis in a 1.5% agarose gel. Marker: λ-DNA digested with Eco RI and Hind III. Three separate experiments were carried out, and the representative result is shown. Reprinted from Kizaki et al.[65] by permission of *Biochemical and Biophysical Research Communications*.

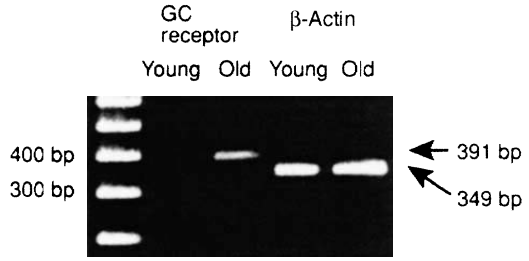

Fig. 5 Expression of GC receptor mRNA on peritoneal exudate cells from young and old mice. Representative results from two separate experiments are shown. The expression of GC receptor and β-actin mRNA in peritoneal exudate cells from young and old mice were analyzed by RT-PCR. Reprinted from Kizaki et al.[73] by permission of *Immunology*.

populations with aging. Thus, we focused our investigation on the age-related changes of the cells of monocyte/macrophage lineage.

The functions and surface phenotypes of peritoneal cells in the monocyte/macrophage lineage from old mice (22–24 month old) were investigated. Proliferative responses of spleen cells from control mice stimulated with Con A were significantly suppressed by adding peritoneal exudate cells from old mice. Flow cytometry analysis revealed that the proportion of FcγRII^{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^{bright} cells, whereas FcγRII^{dull} did not suppress the Con A responses. Acute cold stress increases the proportion of FcγRII^{bright} suppressor macrophages in peritoneal exudate cells of mice[40]. On the other hand, even under normal, unstressed conditions, there is a significant increase in the basal proportion of FcγRII^{bright} cells with suppressor function in old mice.

The generation of the FcγRII^{bright} cells by acute cold stress is mediated by the action of GC through stimulation of HPA[40]. Meanwhile, numerous studies have examined whether GC concentrations change with age, but have failed to reach consensus. Sapolsky[29] analyzed in detail the data obtained from these studies on aging and GC, and concluded that if rats are studied under truly basal, nonstressed circumstances, there is a marked increase in circulating corticosterone concentrations with age throughout the circadian cycle. We also showed that serum concentrations of corticosterone in old mice were markedly higher than those in young mice, supporting the conclusion by Sapolsky[29]. It is thus conceivable that the increase in basal GC concentrations affects 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 mRNAs for GC receptor and β-actin (as a control). Regardless of the almost uniform expressions of mRNA for β-actin, old mice showed high expression of mRNA for GC receptor exceedingly in peritoneal exudate cells as compared with that in young mice (Fig. 5)[73]. Unfortunately, further analysis of GC receptor mRNA expression in terms of cell populations could not be done 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^{bright} cells was analyzed by administering of RU 38486. The treatment with RU 38486 normalized, i.e., rejuvenated the proportion of FcγRII^{bright} cells in peritoneal exudate cells from old mice. These results suggest that the generation of FcγRII^{bright} cells in old mice is mediated by the action of GC through the GC receptor.

The suppressor macrophages induced by acute cold stress strikingly suppress Con A responses of spleen cells from control mice by releasing NO[35]. Also, we observed that accumulation of nitrite in the LPS-stimulated culture supernatants of peritoneal exudate cells from old mice were significantly higher than those from young mice. Further, hypersecretion of NO by macrophages obtained from aged mice following in vitro or in vivo stimulation with LPS was demonstrated[75,76]. Thus the suppression of Con A responses of spleen cells by FcγRII^{bright} macrophages from aged mice might be attributable, in part, to NO.

Effects of aging on immune functions in rats[77,78]

The proportion of cells expressing major histocompatibility complex (MHC) class II molecules and substantial antigen presenting ability in peritoneal monocytes/macrophages from old rats were markedly lower than those from young rats. These findings suggest that the decreased capacity of antigen presentation in peritoneal monocytes/macrophages from old rats is attributable to the low proportion of cells expressing MHC class II molecules. In addition, upon stimulation with LPS IL-1β and IL-6 productions by peritoneal monocytes/macrophages from old rats were markedly lower than those from young rats, implying that functional monocytes/macrophages in peritoneal cells were markedly reduced in old rats.

Analysis of the cell surface phenotype revealed that the number of ED2^{high} cells was markedly decreased in peritoneal cells from old rats as compared with young rats (Fig. 6)[77], although the

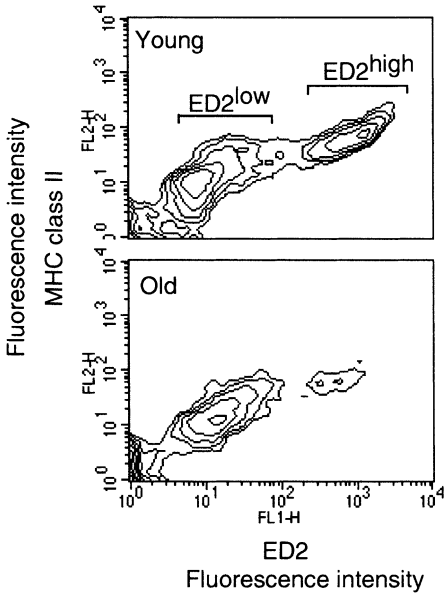

Fig. 6 Two-color flow cytometric analysis of MHC class II and ED2 on peritoneal cells from young or old rats. Peritoneal cells were stained with mAbs ED2 and OX6 and analyzed by flowcytometry after excluding lymphocytes/granulocytes by forward and side scatter. Representative results from four rats each are shown. Reprinted from Kizaki et al.[77] by permission of *Journal of Leukocyte Biology*.

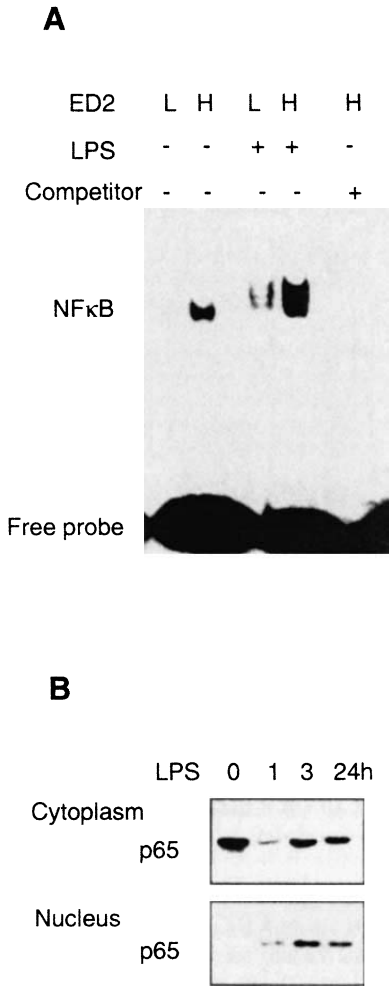

Fig. 7 NFκB activity in ED2^{high} or ED2^{low} cells. A, NFκB DNA binding activity in nuclear extracts of ED2^{high} (H) or ED2^{low} (L) cells was detected by elctrophoretic mobility shift assay. Unlabeled NFκB oligonucleotide probe (competitor) was added (right lane). Representative results of three separate experiments are shown. B, Time course of cytoplasmic and nuclear p65 after stimulation with LPS. ED2^{high} cells were stimulated with 1 μg/ml of LPS for 1, 3, and 24 h. Cytoplasmic and nuclear extracts were prepared and subjected to Western blot analysis with anti-p65 antibody. Reprinted from Kizaki et al.[77] by permission of *Journal of Leukocyte Biology*.

expression of ED1, ED3, or ED8 appeared to be unaffected by aging. We demonstrated that there was no significant difference in the expression of MHC class II molecules between ED2^{high} cells from young and old rats and that the amount of MHC class II expression was significantly higher in ED2^{high} cells than in ED2^{low} cells (Fig. 6)[77]. Efficiency of antigen presentation depends, in part, on the density of MHC class II molecules on the surface of antigen presenting cells. Actually, we were able to demonstrate that the efficiency of antigen presentation resided in ED2^{high} cells but not in ED2^{low} cells, although there was no significant difference in the efficiency of antigen presentation between ED2^{high} cells from young and old rats. IL-1β and IL-6 productions by ED2^{high} cells stimulated with LPS were apparently higher than those in ED2^{low} cells treated with LPS. Furthermore, NFκB activity was extremely higher in ED2^{high} cells than in ED2^{low} cells before as well as after stimulation with LPS (Fig. 7)[77]. These findings indicate that ED2^{high} cells are in a functionally high state. It seems likely, therefore, that the reduced function observed in peritoneal monocytes/macrophages from old rats was attributable not to a decrease in their function but to a decrease in functionally active ED2^{high} cell number.

Regrettably, function and structure of ED2 have not been elucidated[79]. In our preliminary experiments, in vitro phosphorylation of 96-kDa molecules immunoprecipitated with mAb ED2 was observed in an immune complex kinase assay. Thus, ED2 may be involved in signal transduction during cell differentiation or during exhibiting function(s). To elucidate the relationship between ED2 expression and several functions of macrophages, however, further studies are needed.

Cells of the immune system are highly specialized to respond rapidly to diverse unpredictable extracellular events and therefore benefit from using fast-responding, pleiotropically acting triggers at their defense programs. NFκB may play a pivotal role in cells of the immune system because it is rapidly activated by a wide variety of pathogenic signals and functions as a potent, pleiotropic transcriptional activator. Stimulation of monocytes and macrophages with LPS leads to a rapid and transient expression of genes encoding various cytokines. The genes encoding IL-1β and IL-6 have been shown to be good candidates for being induced with the help of NFκB. Nuclear translocation of NFκB in ED2^{high} cells after stimulation with LPS was remarkably higher than that in ED2^{low} cells. By contrast, p65 was present in a large amount in the cytoplasm of ED2^{high} cells in a resting condition, but the cytoplasmic p65 decreased rapidly after stimulation with LPS. Further, p65 was detected in nuclear extract after stimulation with LPS for 1 h. These observations suggest that, in ED2^{high} cells, a large amount of inactive NFκB is retained in the cytoplasm and is rapidly activated after stimulation with LPS, probably leading to the rapid production of large amounts of IL-1β and IL-6.

Meanwhile, several mechanisms of GC-mediated repression of NFκB-dependent gene expression have been proposed[80–86]. We demonstrated that the increase in GC concentrations affects macrophage functions in mice[40]. Eisen et al.[87] reported that GC receptor protein and its mRNA levels were increased following

treatment of human T-cells with GC. The expression of mRNA for GC receptor is enhanced in peritoneal cells from old rats compared with young rats. It thus seems that the stimulation of GC receptors by circulating GC is stronger in old rats than in young rats, which may affect proportions of the peritoneal cells.

Actually, ADX led to a significant increase in the proportion of $ED2^{high}$ cells in the peritoneal cells. The observations obtained suggest that the proportion of $ED2^{high}$ and $ED2^{low}$ cells in peritoneal cells is regulated at least partly by GC, although synergistic effects of the other mediators have not been analyzed in our study. It should be noted that nuclear translocation of the GC receptor was observed in $ED2^{low}$ cells, while markedly higher amounts of GC receptors were retained in the cytoplasm of $ED2^{high}$ cells. Several reports suggest that down-modulation of NFκB-driven genes results from a physical association between activated GC receptors and the NFκB subunit p65[88,89]. It seems, therefore, likely that the GC receptor activation observed in $ED2^{low}$ cells accounts for the repressed functions described above. These findings, however, do not directly explain the decrease in $ED2^{high}$ cells in old rats, since effects of ADX on old rats could not be examined. Therefore, it would not probably be denied that some other or additional immunosuppressive effect may be at work.

Conclusions

We analyzed immunological responses in mice exposed to cold stress. We demonstrated that the immune system of such mice was severely influenced during acute cold stress and cold acclimation. It was somewhat surprising that adherent cells (representatives of cells of mononuclear phagocyte lineage) played an inhibitory role in immunological responses at the early stage of cold stress (acute cold stress), but an accelerative role at the later stage (cold acclimation). The major new findings are that acute cold stress increases MAC-1^{+}FcγRII^{bright} activated macrophages which can exert prominent suppressor functions in peritoneal exudate cells. In addition, we have demonstrated that the generation of the suppressor macrophages is mediated to a greater or lesser degree by increased GC levels following the activation of the HPA by acute cold stress.

Moreover, it has recently been postulated that suppressed cytokine production by monocytes and dendritic cells associated with endotoxin tolerance may result in an inability to respond appropriately to secondary infections[90]. An adaptive immune response is dependent on the presence of antigen receptors displayed on specialized cells of the immune system. However, signals produced by the innate immune system may provide information about the origin or harmfulness of foreign substances and thus determine the kind of adaptive response that is generated[91,92]. Thus, an effective adaptive response generally requires participation of innate response mediators. It appears, therefore, that the FcγRII^{bright} cells from acute cold-stressed mice play an important role at the early stage of infection, but will be unwanted cells later, if they are not able to effectively participate in the subsequent adaptive immune responses. Apoptosis may be an essential mechanism for the homeostasis of immune system: that is, acute cold stress increases activated macrophages which may function in an innate immune response and these macrophages are then eliminated by apoptosis probably for the homeostasis of immune system.

We also analyzed age-related immunosenescence, focussing on the cells in monocyte/macrophage lineage. MAC-1^{+}FcγRII^{bright} suppressor macrophages increased substantially in peritoneal exudate cells from old mice, and the generation of the suppressor macrophages was mediated partly by the increase in basal GC concentrations, probably resulting from dysfunction of the HPA with aging. On the other hand, the number of $ED2^{high}$ macrophages at a functionally high level decreased with age in rats, probably leading to a decline in immune responses. It was also suggested that the proportion of $ED2^{high}$ and $ED2^{low}$ macrophages is regulated, at least in part, by the serum GC concentrations. The differences in immunosenescense between rats and mice can not be discussed, because these experiments were performed using different antibodies against each cell surface molecule. Also, for this reason, populational and functional comparison of MAC-1^{+}FcγRII^{bright} cells with MHC $II^{+}ED2^{high}$ cells cannot be made. These findings appear not to deny the possibility that suppressor macrophages are increased in aged rats, whereas antigen presenting cells are decreased in aged mice.

Aging 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[28]. GC are now recognized to be among the most central of hormones secreted in response to stress after activation of HPA and are considered to be of critical importance in controlling homeostasis[93]. On the contrary, excessive exposure to high GC concentrations will bring about numerous stress-related diseases[94]. HPA dysfunction is often observed with aging. Thus, the changes in GC regulation with aging and the pathogenic consequences of such dysregulations have long been speculated[29,95]. For example, the following questions cannot be answered at present; i.e., whether aged animals respond appropriately to stress, or whether the concentration of GC during basal state, the state of understress and that of post-stress 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 GC concentrations. We have thus demonstrated that macrophages may account for some of the stress- or age-related declines in immune functions and that the stress- and age-related increases in GC concentrations affect macrophage functions. Further studies will be needed to clarify the underlying mechanisms by which neuroendocrine products modulate immune responses and alter disease susceptibility/severity in stressed or aged animals.

Acknowledgements

This study was supported by grants from the Meiji Life Foundation on Health and Welfare, the Nakatomi Foundation, the Daiwa Securities Health Foundation, the Promotion and Mutual Aid Corporation for Private Schools of Japan, and the Japanese Ministry of Education, Science, Sports, and Culture.

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