	くしゃみ・鼻漏型	鼻閉型・充全型	
初期療法	①遊離抑制薬　②抗ヒ薬　③抗 LTs 薬 ④Th2 阻害薬　⑤抗 PGD_2/TXA_2 薬		特異的免疫療法 / 抗原除去・回避
軽　症	①抗ヒ薬　②鼻ステ　③点眼薬		
中等症	抗ヒ薬＋鼻ステ（＋点眼）	抗 LTs 薬＋鼻ステ＋抗ヒ薬（＋点眼）	
重　症 最重症	鼻ステ＋抗ヒ薬＋点眼	鼻ステ＋抗 LTs 薬＋抗ヒ薬＋点鼻血管収縮薬＋点眼薬	
	経口ステロイド薬・手術		

図 3.　花粉症の治療.

（文献 1 より引用改変）

これらの薬剤はアレルギー性鼻炎の治療薬として以前から用いられてきたものであるが，今回の改訂で初期療法においてもその位置づけが明らかにされたと考える.

第 2 世代抗ヒスタミン薬はアレルギー性鼻炎の治療で広く使用されている薬剤で内服薬として 12 種類の薬剤があり，さらに，後期第 2 世代抗ヒスタミン薬として，エピナスチン塩酸塩，エバスチン，セチリジン塩酸塩，ベポタスチンベシル酸塩，フェキソフェナジン塩酸塩，オロパタジン塩酸塩，ロラタジンの 7 剤ある．日常診療で選択の幅が広がり，アレルギー性鼻炎の治療が行いやすくなった半面，一人一人の患者にとってどの薬剤がもっとも適切か，あるいは他の薬剤との相互作用はないかなど，その選択に苦慮することも少なくない．そこで，第 6 版には，これらの抗ヒスタミン薬は分子量や脂溶性などの性質の違いにより中枢神経への移行が異なり中枢神経抑制作用に差があること，アルコール摂取やエリスロマイシン，中枢抑制薬などとの相互作用があることなどが詳細に述べられており，抗ヒスタミン薬を選択する際の参考事項として重要と思われる.

ステロイド薬はアレルギー性鼻炎治療薬の中でもっとも強力な薬剤であり，重症例や最重症例に必要とされることが多い．また，最近，新たな鼻噴霧用ステロイド薬が市販され，ステロイド薬の適応が広がりつつある．そのため，今回の改訂ではステロイド薬が鼻噴霧用ステロイド薬と全身ステロイド薬に分けて表記され，その内容が充実されるとともに，これらの不適切な使用に対する注意が喚起されている.

特異的免疫療法は，その有効性が二重盲検比較試験でも証明され，アレルギー性鼻炎治療薬では不可能な長期寛解を得ることが可能であるが，本邦では必ずしも普及しているとはいえない．そこで，まだ臨床試験段階で保険適応はなく，一般的治療ではないが，将来の特異的免疫療法の一つとして大きな期待が寄せられている舌下免疫療法に関する情報が記載された．舌下免疫療法は海外ですでに多くの臨床研究が行われ，最近，本邦からも優れた臨床試験成績が報告された[2]．本療法の早期の臨床使用が望まれるとともに，これによって特異的免疫療法が見直されることが予想される.

その他，第 6 章では，妊娠へのアレルギー性鼻炎用薬剤投与のリスクに関するオーストラリアと米国の評価基準が変更され，妊婦の薬剤以外の治療法，内服薬や局所用剤による治療における留意点を具体的に記した患者への説明文書の 1 例が追加された．また，最近，小児適応を持つアレルギー性鼻炎治療薬が増えているため小児用薬剤の一覧表が刷新され，小児の治療における留意点も加筆された.

最終項には，2007 年 10 月に発刊されたアレル

表1　アレルギー性鼻炎の診断

	問診 →	鼻鏡検査 →	鼻汁好酸球 →	皮膚テスト →	誘発テスト →	抗体定量 →	過敏性検査
過敏症か？	◎	◎	◎	○	○	○	
アレルギーか？	◎	○	◎	○	○	○	
抗原はなにか？	◎			◎	○	◎	
治療方針の決定	◎	◎	◎	◎	○	◎	○

◎：必須検査　　（文献1より引用）

ギー疾患の横断的なガイドラインである「アレルギー疾患診断・治療ガイドライン」[3]にならって，専門医へ紹介するポイントが新たに追加された．アレルギー性鼻炎を専門とする耳鼻咽喉科医はアレルギーに関する知識のほかに，正確な鼻腔所見の把握と局所処置，とくに手術的治療ができる必要があり，この特質を生かす治療が必要と考えられた場合が紹介のポイントとなる．そのひとつが鼻閉の強い症例や保存的治療によって鼻閉が改善しない症例である．その原因としては，下鼻甲介に不可逆的変化をきたしていること，慢性副鼻腔炎や鼻茸，鼻中隔弯曲症，肥厚性鼻炎，腫瘍などを合併していることが考えられる．したがって，アレルギー性鼻炎で鼻閉が強い症例では鼻鏡検査を行ってこれら疾患を鑑別する必要がある．

また，家族歴や気管支喘息の合併など，アレルギーの素因が強いと思われる小児では，特異的減感作療法を行うことが推奨されている．特異的減感作療法は唯一治癒を導き出せる治療法で，将来的な気管支喘息発症の予防にもつながることがその理由として明記された．

2. ガイドラインのポイントと問題点

アレルギー性鼻炎は発作性反復性のくしゃみ，水様性鼻汁，鼻閉を3大症状とする疾患であり，問診だけでもおおよその診断は可能なように思われる．しかし，ウイルス性の急性鼻炎や老人性鼻漏，さらには血管運動性鼻炎や好酸球増多性鼻炎などとの鑑別診断は専門医にとっても困難なことが少なくない．また，たとえアレルギー性鼻炎の診断ができても，その治療を行うに際して多種多様なアレルギー性鼻炎治療薬の中から適切な薬剤を選択するのは容易ではない．

以下に，診断と治療におけるポイントと問題点について述べる．

1）診断における留意点

アレルギー性鼻炎を正確に診断するには，アレルギー性鼻炎の発症機序を十分に理解する必要がある．アレルギー性鼻炎は典型的なI型アレルギー疾患であり，IgE抗体と抗原の反応を介して肥満細胞から遊離されるヒスタミンやロイコトリエン（LT）などのケミカルメディエーターが即時相のくしゃみ，水様性鼻汁，鼻閉をもたらす．その後，鼻粘膜局所に浸潤した炎症細胞，とくに好酸球が産生するLTによって鼻閉を主症状とする遅発相の反応がみられる．さらにこの反応を何度も繰り返すことで，鼻粘膜の過敏性が亢進し，抗原非特異的な反応や鼻粘膜の浮腫などの不可逆的な器質的障害がもたらされる．

この病態に基づいて，アレルギー性鼻炎か非アレルギー性かを問診や鼻汁好酸球，鼻粘膜の所見で判断し，皮膚テストや誘発テスト，そして特異的IgE抗体の定量によって発症抗原を診断する．(**表1**) しかし，特異的IgE抗体検査に頼りすぎるのは要注意で，特異的IgE抗体が陽性であっても有症者はその約3分の1であり，正常者でも特異的IgE抗体陰性例はむしろ少数である[4]．(**図4**) したがって，アレルギー性鼻炎の診断はこれらすべての検査結果から総合的に判断すべきであり，単一の検査たとえば鼻汁好酸球や特異的IgE抗

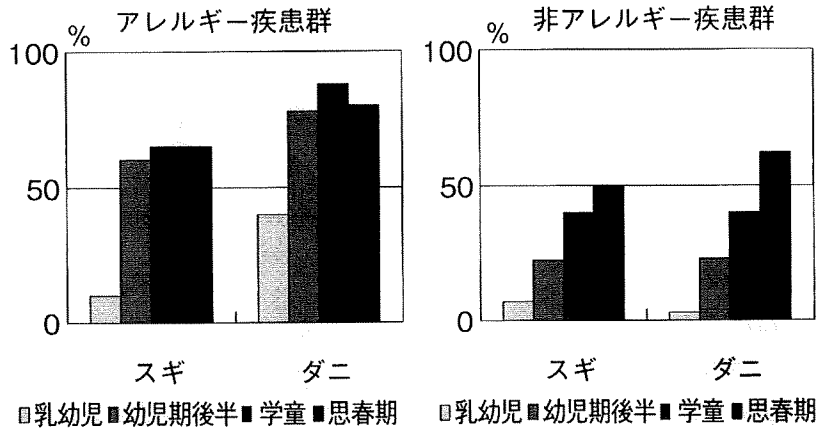

図4. 小児におけるスギ・ダニに対する感作率（CAP-RAST スコアー）.
（文献5より引用改変）

体のみでは他の同様の症状を呈する鼻炎との鑑別は不可能であることを理解する必要がある.

2) 治療における留意点

アレルギー性鼻炎の治療でまず行うべきことは抗原の除去と回避である. しかし, 日常生活のなかでこれを完璧に実施することは不可能であり, 何らかの薬物治療が必要となる. その薬剤も近年次々と新薬が市販され, アレルギー性鼻炎の治療薬が少なく下鼻甲介粘膜切除術などの外科的治療に頼らざるを得なかった時代とは異なり, むしろその選択に苦慮することが少なくない. そこで, ガイドラインには, それぞれの治療薬の特徴を踏まえて, アレルギー性鼻炎の病型と重症度に合わせた薬剤の選択方法が通年性アレルギー性鼻炎と花粉症に分けて示されている. しかし, ここに示されているのはあくまでも初期対応あるいは導入療法であり, 治療経過に応じてどのように薬剤を減量あるいは変更していくのかが明記されていない. 脚注に「症状が改善してもすぐに投薬を中止せず, 数カ月の安定を確かめてステップダウンする」とのみ記されているが, これはかつてケミカルメディエーター遊離抑制薬しかなかったころの記述がそのまま残されているものである. 今後, 導入療法に続く維持療法, 治療効果や投薬終了の判定方法とそのタイミングなどについても何らかの指針を示し, 漫然と長期にわたって薬剤が投与されるのを防ぐ必要があると考える.

花粉症, なかでもスギ花粉症に対してはすでに多くの施設で初期療法が実施されているが, 2005年度版のガイドラインには,「花粉飛散開始とともに, または症状が少しでも現れた時点で薬物療法を開始する. 重症度を念頭においてケミカルメディエーター遊離抑制薬, 第2世代抗ヒスタミン薬, 抗ロイコトリエン薬を選択する.」と記述されているのみで[5], 薬剤によって効果発現時期が明らかに異なるにもかかわらず, その投薬開始時期などが明確に記されていなかった. そこで, この第6版では「例年, 強い花粉症状を示す症例では初期療法を勧める. 第2世代抗ヒスタミン薬は花粉飛散予測日または症状が少しでも現れた時点で内服を開始し, その他の薬剤では飛散予測日の1〜2週間前をめどに治療を始める.」と, その使用法が具体的に記載されている. しかし, 第2世代抗ヒスタミン薬以外の薬剤もそれぞれに薬効や薬理作用が異なり, これらを「その他の薬剤」としてまとめることには問題がある.

また, 今回の改訂で初期療法の薬剤としてTh2サイトカイン阻害薬と抗PGD2・TXA2薬が新たに加えられたが, 鼻噴霧用ステロイド薬はその使用頻度が高いにも関わらず除外されている.(**図2**)その理由として, 初期療法における鼻噴霧用ステロイド薬のエビデンスが乏しいことがあげられる. しかし, 最近, 鼻噴霧用ステロイド薬と抗ヒスタミン薬との非ランダム化オープンラベル並行

群間比較試験が実施され，鼻噴霧用ステロイド薬が第2世代抗ヒスタミン薬より効果が優れることが実証されている[6]．また，アレルギー性炎症が進行するとグルココルチコイド受容体の発現が抑制されるため，ステロイド薬が効きにくくなることも指摘されている[7]．すなわち，花粉症のようにアレルギー性炎症が強い疾患では早期にステロイドを投与するほうが効果的と考えられ，今後，鼻噴霧用ステロイド薬の初期療法薬としての有用性とその作用機序を検証し明かにする必要がある．また，第2世代抗ヒスタミン薬や抗LT薬，Th2サイトカイン阻害薬そして抗PGD2・TXA2薬との使い分けや具体的な投与方法も，このガイドラインに盛り込まれることが切望される．

アレルギー性鼻炎の治療でもっとも難渋するのが鼻閉であり，鼻閉の正しい診断には鼻鏡検査が必要であることは前述した．しかし，耳鼻咽喉科以外の医師がこれを行うのは一般的でなく，このガイドラインが耳鼻咽喉科以外の医師も対象として作成されたという趣旨とはやや矛盾する．しかし，最近は経鼻内視鏡が普及してきており，たとえば，アレルギー性鼻炎や鑑別を必要とする疾患の典型的な鼻腔所見を図示あるいはCD-ROMに挿入するなどすれば，一般医の参考になると思われる．

まとめ

アレルギー性鼻炎診療を向上させるためには，その裾野を広げかつその質の向上を目指すことが必要である．そのためには，このガイドラインを耳鼻咽喉科医のみでなく，より多くの内科医や小児科医の意見をもっと取り上げて，普遍性のあるものへと進化させなければない．これによって，一般の耳鼻咽喉科医そしてアレルギー性鼻炎を専門とする耳鼻咽喉科医の独自性もより認識され，さらにその専門性も高まっていくと考える．

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ORIGINAL ARTICLE

Aging Exacerbates Restraint Stress-Induced Inhibition of Antigen-Specific Antibody Production in Mice

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ABSTRACT

Background: We have recently found that exposure to acute restraint stress suppresses antigen-specific antibody production, including IgE, in a murine model of allergic rhinitis. Although age-related alterations in immune responses are known, it remains unclear whether aging modulates the antibody production under stressful conditions. In this study, we set out to determine the effects of aging on antibody production under acute restraint stress in mice.

Methods: Both young and aged CBA/J mice were repeatedly sensitized intranasally with phospholipase A2 (PLA2) without adjuvants. Restraint stress was applied using uniform cylinders once a week for a continuous 8 h period, on 5 occasions in total. Blood samples were taken at 0, 20 and 30 days after primary sensitization, and production of PLA2-specific antibodies and levels of IL-4, IFN-γ, IL-10 and IL-1β in sera were determined by ELISA.

Results: Repeated intranasal sensitization with PLA2 induced PLA2-specific IgE, IgG1 and IgG2a production in aged mice. We found that exposure to restraint stress significantly inhibited production of PLA2-specific IgE, IgG1 and IgG2a in aged mice. In addition, antibody production under restraint stress decreased significantly in aged mice when compared with young mice. No IL-4, IFN-γ, IL-10 or IL-1β were detected in sera from non-stressed or stressed aged mice.

Conclusions: Aging exacerbates the immunosuppressive role of acute restraint stress in antigen-specific antibody production in mice.

KEY WORDS

aged mouse, immunosuppression, phospholipase A2, restraint stress, specific antibody

INTRODUCTION

It is known that aging is associated with a reduced immune function, so called immunosenescence, in both humans and animals.[1-4] For example, a shift in lymphocyte population from conventional T cells to NK cells and extrathymic T cells is observed in human centenarians.[1] Changes in the proportion of T cell subsets, in addition to increases in memory T cells, impairment of response to mitogens and other stimuli, and alterations in cytokine production also occur with aging.[2-4]

In terms of humoral immunity, it is known that pro-B cells in old mice are impaired in their capacity to rearrange themselves to both D to J and V to DJ gene segments in mice.[5] In addition, serum IgE levels and antigen-specific IgE production are known to decline with age in humans.[6,7]

Exposure to physical, neurological, or emotional stress can also affect both innate and acquired immune responses.[8-10] For example, exposure to acute stress modulates antigen-specific T cell responses.[11] We have recently reported that inhibition of antigen-specific antibody production was confirmed using a type of restraint stress following intranasal sensitization with phospholipase A2 (PLA2) in mice.[12] How-

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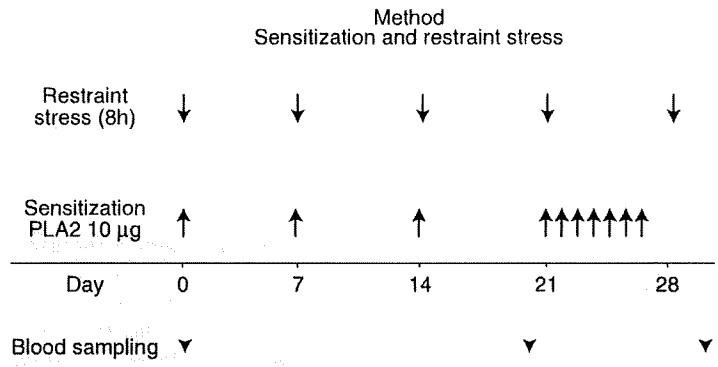

Fig. 1 Treatment schedule; Mice were intranasally sensitized to 10 μg of PLA2 in 20 μl saline. Sensitization was repeated in the same manner. Following sensitization, restraint stress was applied to mice using a single transparent cylindrical chamber and repeated once every week, for a total of 5 applications. Blood samples were taken from each tail vein at 0, 20, and 30 days after primary sensitization.

ever, little is known whether aging affects stress-induced alterations in humoral immune responses.

In this study, we compared stress-induced inhibitions of antibody production between aged and young mice in an intranasal sensitization model. As physical restraint is occasionally used in geriatric care in order to prevent bed fall in hospitals,[13] the results presented here may provide a basis for evaluating the risk of restraint stress on humoral immunity in elderly patients.

METHODS

ANIMALS

Nine-week old female, young adult mice (18–20 g) and 17- month old female, CBA/J strain mice (26–30 g) (Charles River Japan, Yokohama, Kanagawa, Japan) were used in this study. Mice were maintained in an animal house according to the guidelines of the Animal Study Committee of the Kagawa Prefectural College of Health Sciences. All animals were housed in groups of 3, each in an opaque polycarbonate mouse cage (30 × 20 × 30 cm) with access to food and water ad libitum, and were maintained on a 12-hour light-dark cycle for 2–3 weeks before the experiments began. The temperature in the animal house was maintained at 25℃.

REAGENTS

ELISA plates were purchased from Corning (Corning, NY, USA). Purified rat anti-mouse IgE was purchased from Biosource (Camarillo, CA, USA), extraAvidin-peroxidase conjugate, PLA2, carbonate buffer and fetal calf serum from Sigma (St. Louis, MO, USA), tetramethylbenzidine substrate from Kirkegaard & Perry Laboratories (Gaithersburg, MD, USA), phospholic acid from Wako Pure Chemical Industries (Osaka, Japan), peroxidase-conjugated goat anti-mouse IgG1/IgG2a monoclonal antibody from Boehringer-Mannheim (Indianapolis, IN, USA) and biotin (long-arm) N-hydroxy succinimide ester from Vector Laboratories (Burlingame, CA, USA). It is known that endotoxin contamination suppresses allergen-induced immunologic responses including IgE Production on mice.[14] Contamination of endotoxin was negligible as determined using an Endospec assay kit (Seikagaku Kogyo, Tokyo, Japan) in accordance with the manufacturer's instructions.

SENSITIZATION OF MICE

Mice (n = 6–8 per group) were sensitized by nasal administration of 20 μl of saline containing 10 μg of PLA2 using a microsyringe (Hamilton, Reno, NV, USA). PLA2 was carefully given as 7–8 drops of aqueous solution into each nostril in turn. Sensitization was repeated in the same manner after 1 and 2 weeks. On day 21 and on the following 7 consecutive days, the same amount of PLA2 was given in the same manner. Blood samples were taken from the tail vein on days 0, 20, and 30 after primary sensitization (Fig. 1).

INDUCTION FOR RESTRAINT STRESS

Following sensitization, restraint stress was applied to mice (n = 6–8 per group) using a single transparent polymethylmethacrylate cylindrical chamber (20 mm diameter, 100 mm long) commonly used for drawing blood from mice. This chamber was placed horizontally in the mouse cage, and the mice were maintained therein for a continuous 8-hour period without food or water. This manipulation was performed once a week, on a total of 5 occasions (Fig. 1). Control mice were maintained in their cages without food and water at the same time. Three separate experiments were performed to confirm reproducibility.

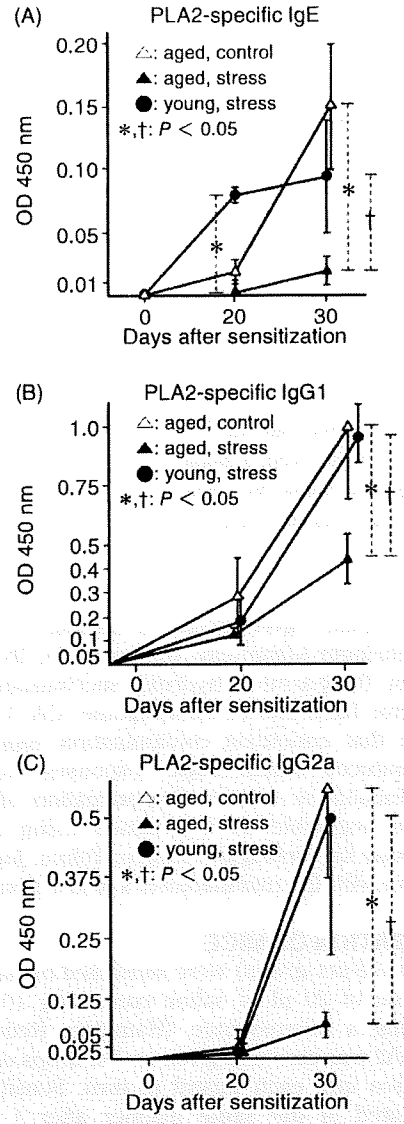

Fig. 2 Effect of restraint stress on PLA2-specific IgE (**A**), IgG1 (**B**) and IgG2a (**C**) production in aged and young mice. Both aged ($n = 9$, closed triangle) and young ($n = 9$, closed circle) were placed in a cylindrical chamber for a continuous 8-hour period without food or water. This manipulation was performed once a week, on a total of five occasions. Control aged mice ($n = 9$, open triangle) were maintained in their cages without food and water at the same time. Blood samples were taken on days 0, 20 and 30 after primary sensitization, and levels of PLA2-specific antibodies were determined by ELISA. Results are expressed as mean ± SEM. Data are representative of 2 separate experiments. $*P < 0.05$ between stressed aged group and control aged group. $\dagger P < 0.05$ between stressed aged group and stressed young group.

PLA2-SPECIFIC IgE, IgG1, AND IgG2a IN SERUM

Serum levels of PLA2-specific IgE, IgG1 and IgG2a were determined using ELISA.[12,14] Titers for specific IgE were estimated as mean optical density (OD) at 450 nm of 1 : 4 diluted sera. Titers for specific IgG1 and IgG2a were estimated as mean OD at 450 nm of 1 : 100 diluted sera.

TOTAL IgE, IgM, AND IgG IN SERUM

Serum levels of total IgE in serum were measured as described previously.[14] The detection limits of this system was 0.3 ng/ml. The levels of total IgM and total IgG were measured using ELISA Quantitation Kit (Bethyl Lavoratories, Inc., Montgomery, TX, USA). The detection limits for IgM and IgG in this system were 0.4 and 0.4 ng/ml, respectively.

CYTOKINE DETERMINATION

Concentration of IL-4, IFN-γ, IL-10 and IL-1β in sera were measured using Opt EIA sets (Becton Dickinson Biosciences, Franklin Lakes, NJ, USA). The detection limits for IL-4, IFN-γ, IL-10 and IL-1β in this system were 10, 60, 15 and 30 pg/ml, respectively.

STATISTICAL ANALYSIS

Data are expressed as means ± standard error of the mean (SEM) for each subject group. Statistical analysis was performed using Student's unpaired t- test to compare titers of PLA2-specific IgE, IgG1 and IgG2a for restrained and control groups. Values of $p < 0.05$ were considered to indicate a statistically significant difference.

RESULTS

EFFECT OF RESTRAINT STRESS ON ANTIGEN-SPECIFIC ANTIBODY PRODUCTION IN AGED MICE

Production of PLA2-specific IgG1 was seen 20 days after the first intranasal sensitization in control aged mice, and production of PLA2-specific IgE and IgG2a, 30 days after the first sensitization. In aged mice under restraint stress, impaired production of these 3 antibodies was observed. On day 30, aged mice under stress produced significantly lower amounts of PLA2-specific IgE, IgG1 and IgG2a as compared with non-stressed aged mice ($P < 0.05$) (Fig. 2A, B, C).

EFFECT OF AGING ON RESTRAINT STRESS-INDUCED INHIBITION OF ANTIGEN-SPECIFIC ANTIBODY PRODUCTION

We then compared PLA2-specific antibody production under restraint stress between young and old mice. Young mice under stress produced PLA2-specific IgE and IgG1 20 days after the first sensitization, and produced PLA2-specific IgG2a 30 days after sensitization. The level of PLA2-specific IgE on day 20 was significantly less in aged mice under stress than young mice, and the difference could still be ob-

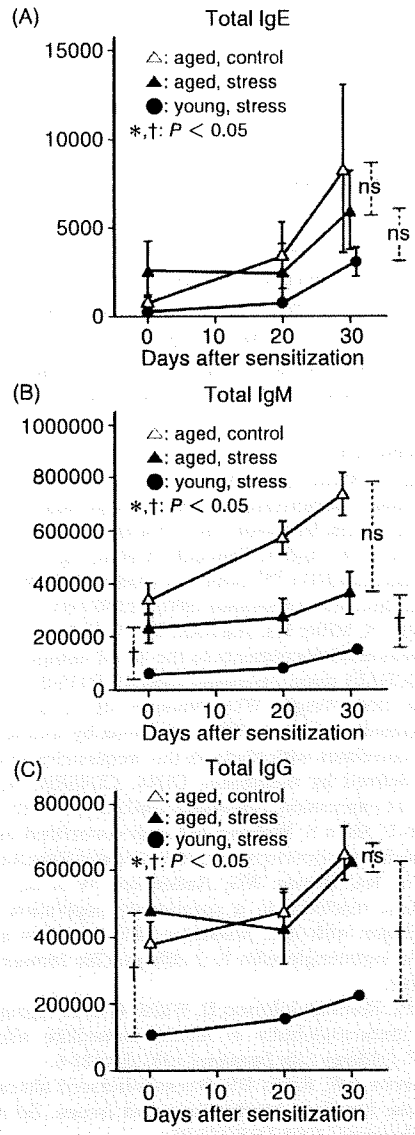

Fig. 3 Effect of restraint stress and/or aging on levels of total IgE (**A**), IgM (**B**), and IgG (**C**) in sera. Both aged (closed triangle) and young (closed circle) mice were placed in cylindrical chambers for a continuous 8-hour period without food or water. This manipulation was performed once a week, on a total of five occasions. Control aged mice (open triangle) were maintained in cages without food and water at the same time. Blood samples were taken on days 0, 20 and 30 after primary sensitisation, and levels of total Ig were determined by ELISA. $*P < 0.05$ between stressed aged group and control aged group. $\dagger P < 0.05$ between stressed aged group and stressed young group.

served on day 30 ($p < 0.05$). In addition, a significant reduction in the production of both PLA2-specific IgG1 and IgG2a was seen on day 30 in aged mice as compared with young mice ($p < 0.05$) (Fig. 2A, B, C).

EFFECTS OF RESTRAINT STRESS ON SERUM LEVELS OF IL-4, IFN-γ, IL-10 AND IL-1β

Serum levels of IL-4, IFN-γ, IL-10 and IL-1β were determined in mice with and without restraint stress. None of these cytokines were detected in sera from non-stressed or stressed aged mice. In addition, these cytokines were not detected even in sera from stressed young mice.

EFFECTS OF AGING AND/OR STRESS ON LEVELS OF TOTAL IgE, IgM, AND IgG IN SERA

Levels of total IgE, total IgM, and total IgG in sera did not differ between stressed aged and non-stressed aged groups. On the other hand, levels of serum total IgM and IgG but not IgE were significantly lower in the stressed young group compared with the stressed aged group throughout the experimental period (Fig. 3).

DISCUSSION

Reductions in T-cell function in aged mice have been shown to reduce IgE antibody production by impairing differentiation of IgE-containing progenitor B cells into IgE antibody-producing plasma cells.[16] These age-associated reductions in immune function, and T-cell function in particular, are thought to affect the function of helper B cells and suppress indirect antibody production response.

In aged mice, various effects of stress in the immune system, and particularly in T cells, have been investigated in previous studies. For example, Kanno *et al.* reported in a study of restraint stress on mice that atrophy of the thymus and decreases in splenic T cells were observed after exposure to stress. However, young mice showed a rapid recovery of the immune function after 1 week, while the aged mice never recovered.[17] However, little is known whether aging can affect stress-induced humoral responses despite the fact that aging and stress share similar effects on immune function.[18]

We previously reported that the humoral immune system in young mice was suppressed by restraint stress in the early stages of antibody production following intranasal sensitization with PLA2.[12] In this study we have further demonstrated that, although repeated intranasal sensitization with PLA2 induced PLA2-specific IgE, IgG1 and IgG2a in aged CBA/J mice, exposure to restraint stress significantly inhibited production of PLA2-specific antibodies. In addition, the present study found that aged mice underwent even more marked suppression of antibody production than young mice under restraint stress. These results suggest for the first time that aging and stress have a synergic effect on the impairment of humoral immunity, and more importantly, that aging exacerbates stress-induced inhibition of humoral re-

sponses. None or only slight differences in antibody production were found between the aged control group and young stressed group. This may be due to an aging effect, and may suggest that the impact of aging on antibody production in our model resembles that of restraint stress seen in young mice.

Restraint stress suppresses both PLA2-specific IgG1 and IgG2a production in aged mice. It is known that IgG1 and IgG2a is Th2 and Th1-type IgG isotype, respectively.[19] Fukui *et al.* reported that restraint stress significantly suppressed both Th1- and Th2-type immune responses in mice.[10] It has also been reported by Dhabhar *et al.* that B cells show a greater stress-induced decrease than T cells.[20] These reports support our findings, suggesting that restraint stress suppresses both Th1- and Th2-type humoral responses in aged mice. Defective induction of functional Th2 cytokine responses has been reported in aged mice[21] in addition to Th1 type immune response being important for the protection against intracellular pathogens such as viruses, mycobacterium and protozoan parasites.[22] Thus susceptibility to impair Th1-type immune responses by restraint stress in elderly patients may increase the risk of suffering from infectious diseases by intracellular pathogens.

The levels of total IgE, total IgM, and total IgG in sera did not differ between stressed aged and non-stressed aged groups. This result suggests that restraint stress selectively affects antigen-specific antibody production in aged mice. Interestingly, levels of serum total IgM and IgG but not IgE were significantly lower in the stressed young group compared with the stressed aged group. This may be due to baseline differences, as serum total IgM and IgG in aged groups were higher than in young groups even before intranasal sensitization. Long-term life in the animal house under a conventional environment may increase serum total IgM and IgG levels.

Although no IL-4, IFN-γ, IL-10 or L-1β was detected in sera from non-stressed aged mice or stressed aged mice, the mechanisms involved in the suppression of antibody production in aged mice under stress have not been clearly elucidated.

Other studies have examined the application of restraint stress, and further studies are needed to clarify the direct or indirect involvement of endocrinological neuronal pathways in the initiation of allergic rhinitis.[23-25] Accumulation of findings from a wide field of research focusing on the immune system and including the nervous endocrine systems is necessary.

In conclusion, we have shown that restraint stress impaired antigen-specific antibody production, especially in aged mice, and aging displays a strong impact on stress-induced inhibition of humoral immune responses. These observations may provide a basis for the management of care for elderly patients with physical restraints. In modern life, both the young and elderly are exposed to various forms of stress.[26] Our study suggests stress as one of the mechanisms for the epidemiological finding that serum IgE levels and antigen-specific IgE production decline with age in humans.[6,7]

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