

IgE regulation and lymphokine patterns in aging humans

Hassan Al-Rayes, MD, William Pachas, MD,^a Nadim Mirza, MD,^a
Deborah J. Ahern, Raif S. Geha, MD, and Donata Vercelli, MD Boston, Mass.

IgE production declines with age, and allergic symptoms tend to improve. Aging therefore represents an in vivo model to study IgE regulation. We compared IgE production in older (≥ 60 years old) and young (15 to 30 years old) nonatopic individuals. Addition of exogenous interleukin-4 (IL-4) to mononuclear cells from older and young subjects induced equivalent amounts of IgE, indicating that IL-4 responsiveness is preserved in aging. After surface receptor stimulation with concanavalin A, IL-4 production by mononuclear cells from older subjects was approximately 50% as compared with the young, whereas interferon- γ (IFN- γ) production was reduced threefold ($p = 0.008$). By contrast, stimulation with phorbol esters and ionophore, which bypass surface receptor signaling, induced comparable amounts of IL-4 and IFN- γ in older and young subjects. These data point to an impairment in T-cell membrane signal transduction in older individuals. This hypothesis was directly confirmed by showing that Ca^{+2} fluxes after CD3 crosslinking were significantly ($p = 0.014$) decreased in the older population. Our findings altogether suggest that an age-dependent T-cell activation defect may result in decreased availability of IL-4 and in the waning of IgE responses. (J ALLERGY CLIN IMMUNOL 1992;90:630-6.)

Key words: IgE, aging, lymphokines, IL-4, IFN- γ , calcium fluxes

IgE production by human peripheral blood mononuclear cells (PBMCs) requires two signals. One signal is delivered by the cytokine interleukin-4 (IL-4), the other by cognate as well as noncognate interactions between T and B cells.¹⁻³ IL-4-dependent IgE production is critically modulated by other lymphokines, such as IL-5,⁴ IL-6,⁵ transforming growth factor- β (TGF- β),⁶ and particularly by interferon- γ (IFN- γ), which strongly inhibits most IL-4-dependent events, including IgE production.^{7, 8} It is interesting to note that IL-4 suppresses IFN- γ production both at the protein and at the mRNA level.^{9, 10} Thus IgE induction by IL-4 is likely to result not only by induction of IgE isotype switching in B cells, but also by an indirect

Abbreviations used

Con A:	Concanavalin A
$\delta\text{nmol/L Ca}^{+2}$:	Rise in intracellular free Ca^{+2}
IFN- γ :	Interferon- γ
IL-4:	Interleukin-4
MAb:	Monoclonal antibody
PBMC:	Peripheral blood mononuclear cells
PMA:	Phorbol myristate acetate
TCR:	T-cell receptor
TGF- β :	Transforming growth factor- β

mechanism, that is, by inhibition of IFN- γ synthesis in T cells.

IgE production steadily declines with age. After reaching peak levels in the latter half of the first decade and at the beginning of the second decade of life, serum IgE levels both in normal and atopic subjects decline progressively to reach the lowest levels after age 60 years.¹¹⁻¹³ In parallel, the incidence of onset of allergic symptoms, as well as their severity, decrease.^{14, 15} Aging therefore represents an interesting in vivo model to study the waning of allergic responses.

We reasoned that the decline of IgE responses observed in aging might result from a decrease in IL-4

From the Division of Immunology, Children's Hospital/Department of Pediatrics, Harvard Medical School, Boston, and Division of Rheumatology, Spaulding Rehabilitation Hospital,^a Boston.

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Reprint requests: Donata Vercelli, MD, Division of Immunology, Children's Hospital, 300 Longwood Ave., Boston, MA 02115.

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responsiveness and/or in IL-4 production and/or from an increased production of IFN- γ , the major IL-4 antagonist. Our data indicate that IL-4 responsiveness was comparable in young (15 to 30 years old) and older (>60 years old) nonatopic healthy subjects. Production of IL-4 seemed to be decreased in the older group, and synthesis of IFN- γ was markedly reduced. This lymphokine production pattern most likely resulted from an age-dependent signal transduction defect. Decreased availability of IL-4 after T-cell receptor crosslinking may lead to the decline of IgE responses observed in aging.

MATERIAL AND METHODS

Population under study

Fifteen older (>60 years old) and 14 young (15 to 30 years old) healthy nonatopic individuals were included in the study. The health status in the older subjects was assessed after the criteria of the SENIEUR protocol for immunogerontologic studies.^{16, 17} Serum IgE levels were 33 ± 19 IU (mean $\pm$ SE) in the young group, and 19 ± 8 IU in the older group (p not significant).

IgE production

Cultures for IgE production were set up as previously described.¹ In brief, PBMCs were isolated by centrifugation on Ficoll Hypaque (Pharmacia, Piscataway, N.J.), resuspended at 1.5×10^6 cells/ml in RPMI 1640-10% fetal calf serum (FCS), and cultured in the presence or absence of IL-4 (Amgen, Thousand Oaks, Calif.) at various concentrations. After 10 days supernatants were harvested and assayed for IgE by RIA. Control cultures for the evaluation of preformed IgE were set up in the presence of cycloheximide (100 μ g/ml; Sigma Chemical Co., St. Louis, Mo.). Net IgE synthesis was evaluated by subtracting the IgE concentrations detected in cycloheximide-treated cultures (always <150 pg/ml) from the IgE values found in untreated cultures.

Lymphokine production

Peripheral blood mononuclear cells (PBMCs) from young and older individuals were cultured in the presence of medium, concanavalin A (Con A, 10 μ g/ml; Calbiochem, La Jolla, Calif.), and phorbol myristate acetate (PMA, 50 ng/ml; Sigma) + ionomycin (0.5 μ mol/L; Calbiochem). PBMCs were cultured at 3×10^6 cells/ml for IL-4 determinations, and at 1.5×10^6 cells/ml for IFN- γ determinations. After 24 hours supernatants were harvested and assayed for IFN- γ by RIA (Centocor, Malvern, Pa.; lower limit of sensitivity, 2 U/ml) and for IL-4 by ELISA (RD Systems, Minneapolis, Minn.; lower limit of sensitivity, 50 pg/ml).

Measurement of intracellular free Ca^{+2} concentrations

Variations in intracellular free Ca^{+2} concentrations after crosslinking of surface receptors were measured as de-

scribed by Rabinovitch et al.¹⁸ In brief, PBMCs were isolated by Ficoll Hypaque density gradient centrifugation and adhered overnight at 37°C in RPMI 1640-10% AB+ (Flow Labs Inc., McLean, Va.). These T-cell-enriched populations were then washed with RPMI 1640, and loaded with the acetoxymethyl ester of indo-1 (1 μ mol/L; Molecular Probes, Eugene, Or.). After a 40-minute incubation at 37°C, the cells were washed, resuspended at 1×10^6 cells/ml, and incubated on ice in the dark. Before stimulation, the cells were equilibrated at 37°C for 2 minutes then incubated with anti-CD3 monoclonal antibody (MAb) (10 mm³ of a 1:500 dilution of OKT3 ascites) followed by goat antimouse Ig (10 μ g/ml; TAGO, Burlingame, Calif.), and analyzed by flow cytometry with use of a FACStar Plus (Becton Dickinson, Mountain View, Calif.) as described.^{18, 19} Results are expressed as rise in intracellular free Ca^{+2} concentrations over background values ($\delta\text{nmol/L Ca}^{+2}$), at the peak of the response.

Statistical analysis

Statistical analysis was performed with use of the Student's t test for unpaired samples.

RESULTS

IL-4 responsiveness is comparable in young and older individuals

To investigate whether a decrease in IL-4 responsiveness underlies the decline in IgE levels observed in aging, we compared PBMCs from 13 young (15 to 30 years old) and 13 older (>60 years old) healthy nonatopic subjects for their ability to synthesize IgE after addition of exogenous IL-4 at different concentrations. IgE production in this system requires an isotype (IgE)-specific signal, delivered by IL-4, and a B-cell activating signal, delivered by cognate interaction between the T-cell receptor (TCR)/CD3 complex on T cells and major histocompatibility complex class II antigens on B cells.¹ Additional, noncognate pathways (e.g., CD40/ligand interactions) might also be involved in triggering IgE production.^{20, 21} Fig. 1 shows that no spontaneous *in vitro* IgE production was detectable in either group, whereas vigorous IgE production was detected after IL-4 stimulation. No statistically significant difference was detected between IgE synthesis in the two groups, even when suboptimal IL-4 concentrations (50 U/ml) were used to uncover subtle differences in IL-4-dependent responses. These results suggest that IL-4 responsiveness, as expressed by the ability to synthesize IgE in response to exogenous IL-4, is not affected by aging.

IL-4 production in aging

Because responsiveness to exogenous IL-4 did not significantly differ in young and older subjects, we then investigated whether the decline in IgE produc-

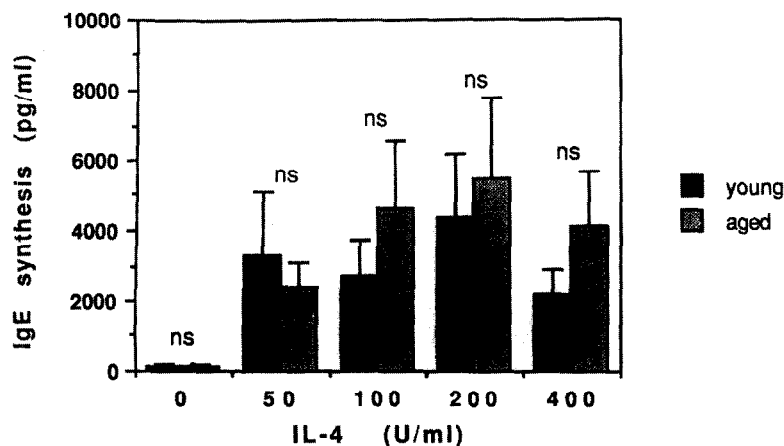


FIG. 1. IgE synthesis by PBMCs from young ($n = 13$) and older ($n = 13$) individuals after stimulation with increasing IL-4 concentrations. Mean $\pm$ SE of net IgE synthesis is shown. *ns*, Not significant.

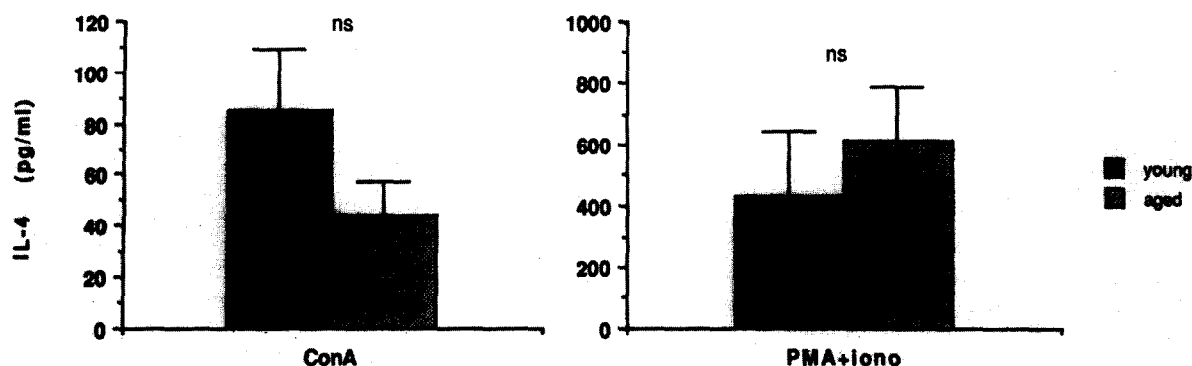


FIG. 2. IL-4 production by PBMCs from young ($n = 14$) and older ($n = 13$) individuals after stimulation with Con A (left panel) and PMA + ionophore (right panel). Mean $\pm$ SE of IL-4 concentrations in culture supernatants is shown.

tion observed in the elderly population resulted from an impairment in IL-4 production. To this purpose, we assessed the ability of PBMCs from 14 young and 13 older individuals to secrete IL-4 after activation by different stimuli. As mitogens, we used either Con A, which delivers an activating signal via surface receptors,²² or PMA + ionophore, which bypass surface receptors and mimic the effects of second messengers.²³ Fig. 2 shows that IL-4 production in cultures stimulated with PMA + ionophore was intense, and comparable in young and older individuals. By contrast, in agreement with previous reports IL-4 production in Con A-stimulated cultures was modest,^{24, 25} indeed close to the lower limit of sensitivity of the assay (50 pg/ml). Although no statistically significant difference was evident between old and young subjects, IL-4 production in the young was approximately twofold greater than in the older group.

IFN- γ production is impaired in aging

The IL-4 antagonist IFN- γ plays a major role in the regulation of IgE synthesis in humans by exerting an inhibitory effect on IL-4-dependent IgE synthesis.^{7, 8} Because our results provided no conclusive evidence for an impairment of IL-4 production in the older population, we asked whether the progressive decline in IgE production observed with aging might be related to an increase in IFN- γ . To this purpose, we tested IFN- γ production by PBMCs from 14 young and 14 older subjects stimulated with ConA or PMA + ionophore. Fig. 3 shows that IFN- γ production by PBMCs stimulated with PMA + ionophore was equivalent in the two groups. In contrast, IFN- γ production by Con A-stimulated PBMCs from older individuals was decreased threefold ($p = 0.008$) as compared with young subjects. The finding of decreased IFN- γ synthesis after stimulation with Con

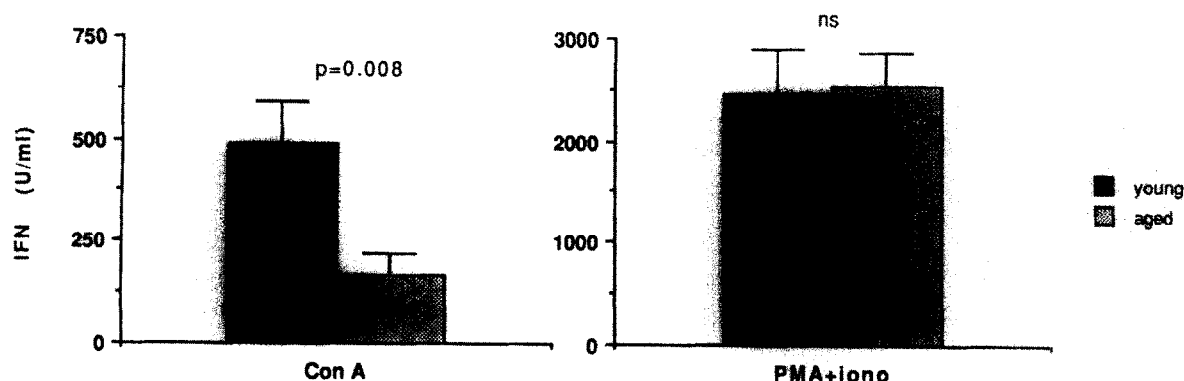


FIG. 3. IFN- γ production by PBMCs from young ($n = 14$) and older ($n = 13$) individuals after stimulation with Con A (left panel) and PMA + ionophore (right panel). Mean $\pm$ SE of IFN- γ concentrations in culture supernatants is shown.

A, but not phorbol esters, strongly suggested that the decrease in IFN- γ production in aging may reflect a defect in T-cell-transmembrane signaling.

Ca²⁺ fluxes in cells stimulated via CD3 are reduced in aging

To directly assess whether a defect in signal transduction through surface receptors underlies the decrease in lymphokine production by T cells from older individuals, we measured a critical event in the signaling cascade, that is, the rise in intracellular Ca²⁺ concentrations after crosslinking of the TCR/CD3 complex. To this purpose, T-cell-enriched populations from 10 young and 12 older individuals were loaded with a highly sensitive fluorescent Ca²⁺ indicator, indo-1. When excited by UV light, indo-1 exhibits large changes in fluorescence emission wavelengths on calcium binding. The use of the ratio of intensities of fluorescence at two wavelengths permits measurement of free Ca²⁺ independent of variability in intracellular dye concentration.^{18, 19} Indo-1 fluorescence was continuously monitored for 10 minutes after the addition of murine anti-CD3 MAb followed by a goat antimouse antibody. Intracellular free Ca²⁺ concentrations in unstimulated T-cell-enriched preparations were comparable in young and older subjects (data not shown). However, after crosslinking of the TCR/CD3 complex, a significantly ($p = 0.014$) lower rise in intracellular free Ca²⁺ (δ nmol/L Ca²⁺) was detected in the older group, as compared with young subjects (Fig. 4). These results confirm the existence of a defect in transmembrane signal transduction in the older population.

DISCUSSION

Youth is the most usual time to develop allergy. Typically, most patients with allergy manifest their

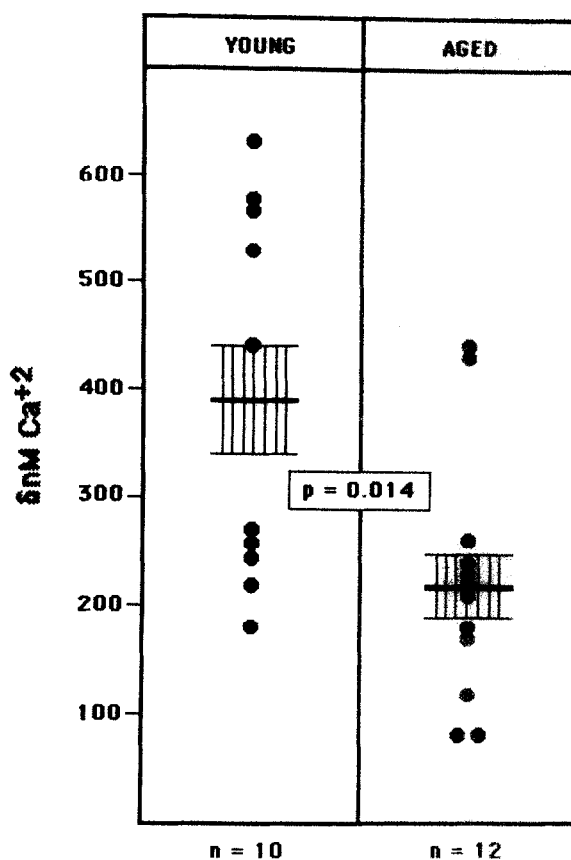


FIG. 4. Rise in intracellular free Ca²⁺ after CD3 crosslinking in cells from young ($n = 10$) and older ($n = 12$) subjects. Bars in hatched area indicate mean $\pm$ SE of δ nmol/L Ca²⁺ (rise in intracellular free Ca²⁺ concentrations over background values, at the peak of the response).

symptoms before age 20 years. With age, detection of allergies becomes less frequent, and symptoms tend to improve.¹⁴ For an IgE response to develop, brisk IL-4 responsiveness and efficient T/B cell inter-

actions are required, together with an appropriate pattern of lymphokine secretion.³ Comparable IgE synthesis was induced in PBMCs from older and young individuals after addition of exogenous IL-4, even at suboptimal concentrations. We can therefore conclude that IL-4 responsiveness of B cells activated by cognate and/or contact-dependent signals is intact in older humans. A decrease in the IgE producing ability of murine B cells stimulated with IL-4 and lipopolysaccharide had been previously reported.²⁶ The discrepancy between these findings and our data is likely to reflect the difference in the activation pathways used to trigger IgE production in human and murine B cells.

Having ruled out an impairment in IL-4 responsiveness, the decrease in IgE production observed in aging may then result from an altered pattern of lymphokine secretion, that is, from a defect in IL-4 production and/or from an imbalance in the IL-4/IFN- γ ratio. Reliable quantitation of IL-4 produced by human lymphocytes activated via physiologic pathways is difficult at the present time because of the limited sensitivity of the assays.^{24, 25} Our data show a twofold decrease in Con A-induced IL-4 synthesis in the older group. This decrease, however, was not statistically significant. In parallel, a threefold decrease occurred in the production of IFN- γ , which was statistically highly significant ($p = 0.008$). IL-4 production by T cells from old mice has been reported to be increased, rather than decreased.^{27, 28} However, the ability to produce lymphokines is differently distributed within human and murine T cells, because IL-4 in mice is selectively produced by Th2 cells, whereas IL-2 and IFN- γ are secreted by Th1 cells.²⁹ In contrast, most human T cells do not fit into the Th1/Th2 dichotomy, and secrete both IL-2 and IL-4.^{30, 31} The observed discrepancy may therefore well derive from the different lymphokine profiles of murine and human CD4⁺ T cells.

Two lines of evidence suggest that the pattern in the secretion of T-cell-derived lymphokines we detected in the older group might result from a defect in signal transduction across the T-cell membrane. First, when surface receptors were bypassed by stimulation with PMA + ionophore, comparable secretion of IL-4 and IFN- γ was induced in older and young individuals. Furthermore, we were able to show decreased Ca²⁺ fluxes in response to CD3 crosslinking in the older group. The existence of an impairment in T-cell activation in aging has long been recognized.³² A defect in the "calcium signal" is central to an intriguing model recently proposed for the immunodeficiency of aging.³³ In young mice some, but not all, T cells flux Ca²⁺ in response to Con A or anti-CD3 MAb.^{34, 35} The T cells that flux Ca²⁺ are CD44^{lo}/CD45R⁺, and represent virgin T cells.³⁶ Be-

cause of thymic involution, the virgin T-cell population that strongly fluxes Ca²⁺ with mitogens declines in aging mice, whereas the Ca²⁺ resistant T-cell subset expands.³⁶ Ca²⁺ resistant T cells have been identified with CD44^{hi}/CD45R⁻ memory cells,³⁶ which are responsible for most IL-4 and IFN- γ production.^{24, 37} Because memory cells, although increased in number, do not readily flux Ca²⁺ after surface stimulation, proliferation and secretion of IL-4 and IFN- γ in response to antigen decline, resulting in immune dysfunction.^{33, 38, 39} Consistent with this hypothesis, an accumulation of CD45R⁻ memory T cells has been observed in aging humans.^{40, 41}

In the context of an age-dependent impairment in the responsiveness to surface-mediated activation signals, a subtle defect in IL-4 may well underlie the progressive decrease in IgE production observed in human aging. Allergen-specific human T cells typically express a Th2 phenotype, that is, they produce high amounts of IL-4.⁴²⁻⁴⁵ Because isotype switching to IgE is critically dependent on IL-4,³ decreased availability of this lymphokine as a result of defective signal transduction could result in reduced IgE induction. The concomitant decrease in the IL-4 antagonist, IFN- γ (caused by the same age-related signaling impairment) may not be sufficient to override the consequences of the IL-4 defect on IgE production.

Alternatively, the possibility should be considered that other factor(s) may also contribute to the waning of IgE responses in aging. IL-6 is known to be required for high-rate production of different Ig isotypes,⁴⁶ including IgE.⁵ Preliminary data obtained on a small group of subjects suggest that IL-6 secretion was not impaired in the older population under study (data not shown). It will be interesting to investigate whether autacoids, such as prostaglandin E₂,⁴⁷ or other cytokines, such as platelet-activating factor⁴⁸ and TGF- β ,⁶ known to modulate IL-4-dependent IgE production, play a role in IgE regulation in the older population.

Finally, this study included only young and older nonallergic individuals. The difference in their serum IgE levels was approximately 50%. It would be of interest to compare cytokine production in allergic older and young subjects in whom the differences in serum IgE levels are more pronounced.

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Arachidonic acid metabolism in monocytes of aspirin-sensitive asthmatic patients before and after oral aspirin challenge

Uwe R. Juergens, MD, Sandra C. Christiansen, MD,
Donald D. Stevenson, MD, and Bruce L. Zuraw, MD *La Jolla, Calif.*

Aspirin and nonsteroidal antiinflammatory drugs induce bronchospastic reactions in patients with aspirin-sensitive respiratory disease. Although the mechanism of this reaction is unknown, all drugs that induce the respiratory reaction also inhibit the cyclooxygenase enzyme. The ensuing changes in arachidonate metabolism are presumed to play a role in the pathogenesis of the reaction. We measured generation of leukotrienes and thromboxane by calcium ionophore stimulated blood monocytes. Before aspirin challenge, monocytes released significantly more thromboxane B₂ in patients with aspirin sensitivity than in patients without aspirin sensitivity or in healthy control subjects ($p < 0.02$). During aspirin-induced bronchospasm, release of leukotriene B₄ increased significantly (45.5%, $p = 0.018$), whereas release of thromboxane B₂ decreased (-46.9%, $p = 0.028$). Two hours after ingestion of 60 mg aspirin, normal monocyte release of thromboxane B₂ did not drop, whereas leukotriene B₄ release increased. Monocytes formed only minimal amounts of leukotriene C₄. We conclude that the profile of released eicosanoids from aspirin-sensitive monocytes is distinct from non-aspirin-sensitive subjects, and that these differences could contribute to the development of bronchospasm after aspirin ingestion. (J ALLERGY CLIN IMMUNOL 1992;90:636-45.)

Key words: Aspirin-sensitive asthma, arachidonic acid metabolism, monocytes, aspirin challenge, thromboxane B₂, leukotriene B₄

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Reprint requests: Bruce L. Zuraw, MD, The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA 92037.

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Approximately 10% to 20% of the asthmatic population is sensitive to aspirin. After ingestion of aspirin or structurally unrelated nonsteroidal antiinflammatory drugs (NSAIDs), asthmatic reactions with or without accompanying nasooocular symptoms characteristically develop in patients with aspirin-sensitive respiratory disease (ASRD).¹⁻⁴ The potency of these compounds in causing bronchospasm is closely associated with the degree of inhibition of the cyclooxygenase enzyme, giving rise to the hypothesis that NSAIDs and aspirin induce these reactions through an alteration in arachidonic acid (AA) me-