Age-related differences in the effect of in vivo administration of indomethacin on hemopoietic cell lineages of the spleen and bone marrow of mice

S. C. Miller

Department of Anatomy, McGill University, 3640 University Ave., Montreal, P.Q. (Canada H3A 2B2)
Received 13 May 1991; accepted 20 February 1992

Abstract. During 21 days of indomethacin treatment, erythroid cells in the spleens of both young adult and older mice, and in the bone marrow of young adult mice, were increased significantly early in treatment, relative to age-matched control organs, and remained high throughout treatment. During drug exposure, the numbers of myeloid cells in young adult bone marrow, but not spleen, were reduced, but in older mice these cells were elevated in both organs. Lymphoid cells in the young adult and older mouse spleens decreased and increased, respectively, during treatment, but were unchanged and decreased, respectively, in the bone marrow of young adult and older mice. Monocyte-macrophage cells in the spleen were elevated but unchanged in the bone marrow of both age groups. During 14 days of indomethacin treatment of young adult mice, the proportions of precursor cells in DNA synthesis of only the splenic erythroid lineage were increased. Thus, the major hemopoietic lineages in both the bone marrow and spleen are affected by exposure to indomethacin in a time-dependent and age-dependent manner. For all lineages studied, those of the bone marrow were least disturbed and/or were first to recover, even during continued drug exposure.
Key words. Indomethacin; hemopoietic cells.

The drug, indomethacin, has recently gained considerable recognition for its beneficial effects in tumor therapy in laboratory animals and man [1–4], especially when used in conjunction with the cytokine, interleukin-2. Indomethacin, in interfering with the cyclo-oxygenase activity, inhibits the production of prostaglandin E_2 (PGE_2)[5, 6], thereby releasing cells of the hemopoietic and immune systems from the suppressive effects of this ubiquitous agent [7–12]. The significant enhancement of natural killer (NK) cells, a major component of the tumor immunosurveillance mechanism, is one result of in vivo administration of indomethacin [6, 13–15].

The effects of in vitro exposure to indomethacin have been studied with respect to cells of both the granulocyte-macrophage lineage [16], and the lymphoid lineage, including T, B and NK cells [14, 17–19]. Other studies have been concerned with the effects of single or multiple injections of the drug in vivo, followed by analyses of the subsequent in vitro behavior of various hemopoietic cells [11, 14, 18, 20]. Fully in vivo studies on the hemopoietic and immune cells following indomethacin exposure have only concentrated on recording changes in whole organ cellularity or circulating white blood cells [11].

The present study aimed to assess in detail the population dynamics of specific hemopoietic cell lineages in young adult and more aged mice. The study includes an analysis of the effect of indomethacin on the proportions of hemopoietic cells in DNA synthesis in the spleen and bone marrow following various periods of drug exposure.

Materials and methods

Mice. Male DBA/2 mice (Charles River Laboratories, St. Constant, Quebec, Canada) were used either at 7–9 weeks of age, or at 16–18 months, having been allowed to age from young adulthood in the Animal Care Facilities of McGill University.

Drug and isotope administration. Mice of both age groups received indomethacin (Sigma Chemical Co., St. Louis, MO) (5 mg dissolved in 1 ml absolute ethanol) via the drinking water, a common vehicle for its in vivo administration [3, 4, 6], in a 1 : 100 ratio. Fresh solution was provided in drinking water every second day. No difference in the amount of water consumed between groups of mice given indomethacin, the indomethacin vehicle (ethanol in water) or untreated drinking water was observed as determined initially by measurements taken at 2-day intervals over the course of 1 week.

Treated mice were killed at 0, 6–7, 14 or 21 days after beginning drug treatment. No visible evidence of hemorrhage, a potential side effect of the drug resulting from interference with the clotting mechanism, was found after stereoscopic examination of the gut from the stomach down to and including the distal bowel, at any sampling interval throughout drug treatment. Half of the young adult mice and all of the older mice were so examined and compared with identical untreated mice. To assess the effect of the drug on the proportions of cells in DNA synthesis in various hemopoietic lineages, drug-treated and control mice of the young adult age group were given a single intra-peritoneal injection of tritiated (^{3}H-)thymidine (1 μCi/g b. wt, spec. act. 20.0 Ci/mM; New England Nuclear Corp., Boston, MA) at 0, 7 and 14 days after beginning drug treatment and killed 1 h after that injection.

Preparation of bone marrow and spleen cells. Free cell suspensions of bone marrow and spleen were prepared as described elsewhere [21–23]. In some experiments, bone marrow and spleen cells were deposited onto gelatin-coated slides in a cytocentrifuge (Cytospin, Shandon Southern Instruments, Inc., Selwicky, PA) and stained with MacNeal's tetrachrome hematologic stain. In other cases, where mice received ^{3}H-thymidine injections prior

to sampling, smears of bone marrow and spleen cells were subsequently fixed in methanol and prepared for radioautography by coating with Kodak NTB_2 liquid emulsion, exposing for 21 days, processing and staining with a modified MacNeal's stain.

Differential analysis of hemopoietic cells. From both the bone marrow and spleen preparations from each animal, 1000–2000 nucleated cells were morphologically identified and enumerated as fully described elsewhere[21]. In the myeloid and lymphoid lineages, cells were grouped according to their stage of maturity. Immature myeloid cells (myeloblasts, promyelocytes and myelocytes) are the well-established proliferative forms while mature cells (metamyelocytes, band and polymorphic forms) are post-mitotic. Immature, proliferating cells of the lymphoid lineage, morphologically and kinetically defined previously by ^{3}H-thymidine-incorporating capacity[21–24], have mean nuclear diameters of $\geq 8.0\,\mu$ in smears or $\geq 10.0\,\mu$ in cytospot preparations. Their mature, non-proliferating progeny, i.e., small lymphocytes[22–24], range from 6.5–8.0 μ in nuclear diameter in smears and $< 10.0\,\mu$ in cytospots. Erythroid precursors include all nucleated elements of that lineage, up to, but excluding the reticulocytes which contain a pycnotic nucleus and cytoplasm characteristic of the mature, non-nucleated, erythrocytes. From smears of the spleen and bone marrow, the proportions of positively (above background) labelled (≥ 3 radioautographic grains) cells of several morphologically distinct subpopulations were recorded. From the known total organ cellularity (spleen, femur) and the proportions of cells in each lineage, the absolute numbers of cells/lineage/organ were obtained.

Statistical evaluation. Student's t-test was used to compare differences between means. $P < 0.05$ was considered significant. The control group for each experimental group was the corresponding value at time 0 of indomethacin treatment.

Results

Nucleated erythroid cells in the spleens of young adult mice increased 3-fold after 6–7 days of drug exposure and remained at twice the control (day 0) levels thereafter (table 1). The numbers of myeloid cells in the spleens of these mice appeared unaffected by drug treatment. On the other hand, the total numbers of lymphoid cells in such spleens fell progressively during prolonged drug exposure to 67% of control values by 21 days of drug exposure. Almost all of the cells categorized as 'other' in the spleen were morphologically identifiable cells of the monocyte-macrophage lineage. The absolute numbers of such cells in the spleen were consistently elevated under drug influence, approximately doubling throughout drug treatment (table 1). In the bone marrow of young adult mice (table 1), erythroid cells were also increased throughout indomethacin treatment, above control (day 0). Myeloid cells in the bone marrow differed little in numbers from those of untreated, young adult bone marrow controls (day 0), the latter, in turn, agreeing with our previously reported values in normal young adult C3H/HeJ mice[21]. Indomethacin had no effect, at any time, on the numbers of lymphoid or monocyte-macrophage cells in the bone marrow of these young adult mice.

Table 2 indicates that the relative proportions of immature and mature cells of the lymphoid lineage in both the spleen and bone marrow of the young adult mouse remained consistent throughout indomethacin treatment even though, in the case of the spleen, the lymphoid cell population, as a whole, was consistently sub-normal (table 1). In the spleens of the young adult mice, the

Table 1. Effect of indomethacin on various hemopoietic cell populations in DBA/2 mice

			Days of indomethacin treatment							
			0		6–7		14		21	
Organ	Age of mice	Cell lineage	%	$\times 10^6$ cells/ organ	%	$\times 10^6$ cells/ organ	%	$\times 10^6$ cells/ organ	%	$\times 10^6$ cells/ organ
Spleen	7–9 wk	Erythroid	12.5 ± 0.9	13.9	31.2 ± 5.3	42.9	25.0 ± 3.1	29.0	28.0 ± 3.2	28.1
		Myeloid	6.1 ± 1.1	6.9	8.4 ± 0.8	11.0	9.1 ± 1.9	10.1	6.9 ± 0.7	7.0
		Lymphoid	78.5 ± 5.9	87.1	55.8 ± 5.1	75.8	61.1 ± 4.9	69.9	58.0 ± 6.6	57.9
		Other	2.9 ± 1.1	3.2	4.6 ± 2.1	6.3	4.8 ± 0.9	5.5	7.1 ± 1.6	6.9
Bone marrow (/femur)	7–9 wk	Erythroid	15.8 ± 2.7	1.8	24.9 ± 3.1	2.7	22.4 ± 3.5	2.7	19.6 ± 2.7	2.3
		Myeloid	56.3 ± 3.1	6.5	45.0 ± 3.9	4.9	49.2 ± 3.7	6.0	50.7 ± 4.6	5.9
		Lymphoid	23.4 ± 3.0	2.7	26.1 ± 3.8	2.8	23.1 ± 2.6	2.8	24.3 ± 1.9	2.8
		Other	4.5 ± 1.1	0.52	4.0 ± 0.9	0.43	5.3 ± 1.6	0.64	5.4 ± 0.8	0.62
Spleen	16–18 mo.	Erythroid	3.1 ± 0.6	2.6	9.0 ± 1.9	10.4	ND	ND	12.4	18.2
		Myeloid	9.8 ± 3.0	8.3	16.6 ± 4.2	19.2	ND	ND	25.0	36.7
		Lymphoid	80.5 ± 6.3	68.1	67.9 ± 7.1	78.5	ND	ND	57.5	84.3
		Other	6.6 ± 1.1	5.6	6.5 ± 2.1	7.5	ND	ND	5.1	7.5
Bone marrow (/femur)	16–18 mo.	Erythroid	16.5 ± 3.6	3.3	12.6 ± 2.4	3.2	ND	ND	13.8 ± 1.9	2.8
		Myeloid	61.7 ± 5.4	12.3	71.9 ± 8.6	17.9	ND	ND	74.1 ± 6.1	15.1
		Lymphoid	17.1 ± 3.0	3.4	10.4 ± 2.6	2.6	ND	ND	6.5 ± 1.2	1.3
		Other	4.7 ± 1.9	0.94	5.1 ± 2.0	1.3	ND	ND	5.6 ± 1.0	1.1

In young adult and older mice, the data represent the mean ± SE of 5–10 and 4–5 animals, respectively. At 21 days, the splenic values from aged mice are based on a pool of 5 mice. ND = not done.

Table 2. Effect of indomethacin on the relative numbers of immature cells in the myeloid and lymphoid cell lineages in the spleen and bone marrow of DBA/2 mice.

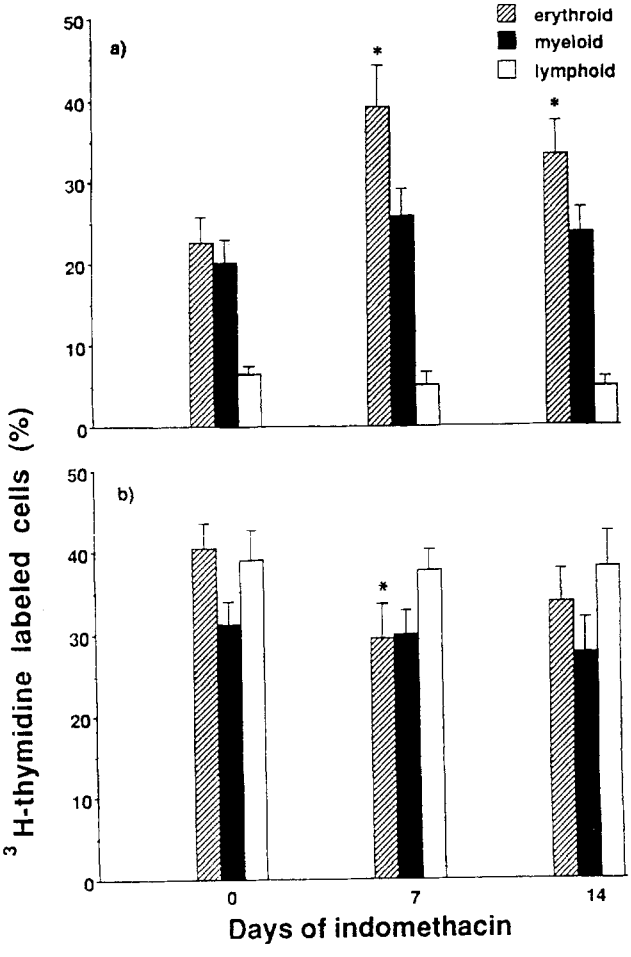
Organ	Age of mice	Cell lineage	Percentage of immature cells after indomethacin treatment for 0 days %	6–7 days %	14 days %	21 days %
Spleen	7–9 wk	Myeloid	42.5 ± 3.2	69.2 ± 3.7*	60.0 ± 2.6*	58.3 ± 3.0*
		Lymphoid	14.3 ± 2.6	20.1 ± 3.8	18.0 ± 1.9	15.9 ± 1.8
Bone marrow	7–9 wk	Myeloid	60.8 ± 3.2	32.7 ± 1.9*	56.9 ± 2.8	67.2 ± 3.1
		Lymphoid	22.9 ± 2.9	25.3 ± 2.0	26.5 ± 3.0	24.3 ± 2.2
Spleen	16–18 mo.	Myeloid	81.1 ± 4.1	78.8 ± 4.6	ND	74.4
		Lymphoid	11.0 ± 3.1	10.1 ± 2.6	ND	11.6
Bone marrow	16–18 mo.	Myeloid	66.5 ± 6.1	59.9 ± 6.3	ND	68.0 ± 5.5
		Lymphoid	14.6 ± 3.4	18.2 ± 3.6	ND	16.9 ± 4.1

Percentage of cells within each lineage which are morphologically immature as defined in 'Materials and methods'. In young adult and older mice, data represent the mean ± SE of 5–10 and 4–5 animals, respectively. At 21 days, the splenic values from older mice are based on a pool of 5 mice. ND = not done. The asterisk indicates a significant difference from control (day 0).

relative numbers (table 2) of immature vs mature cells in the myeloid lineage showed a significant deviation from control ($p < 0.01$), being most pronounced at the earliest treatment interval (6–7 days) assessed. In the young, adult bone marrow, immature cells in the myeloid lineage normally outnumber the mature cells, the latter destined for exit into the blood soon after formation. However, after 6–7 days of drug exposure, immature cells decreased significantly ($p < 0.01$) to almost half their normal relative numbers within the population (table 2). With prolongation of drug exposure in young adult mice, a restoration in the relative numbers of immature myeloid cells occurred in the bone marrow, reflecting a return to normal ratios (day 0, table 2), even during continued drug presence.

Irrespective of changes in population size or in the relative numbers of immature vs mature cells, the proportions of precursors in DNA synthesis in both the lymphoid and myeloid lineages in the spleens of young adult mice were unaffected by the drug (fig.). Of all the immature precursors in the 2 lineages, there was no difference at any treatment period in the proportions of those precursors in DNA synthesis as determined by ^{3}H-thymidine uptake during a 1-h exposure to the isotope (fig.). Similarly, in the bone marrow, precursors in these 2 lineages showed no change in isotope labelling irrespective of drug exposure time (fig.). In both the spleen and bone marrow, however, the DNA-synthesizing erythroid precursors were affected by indomethacin. The proportion of DNA-synthesizing cells (^{3}H-thymidine-labelled) among the erythroid precursors in the spleen was increased ($p < 0.05$) by 7 days of treatment and remained elevated thereafter (14 days). In the bone marrow, on the other hand, the proportions of labelled cells among the erythroid precursors, relatively high in normal (control) mice, fell marginally to 3/4 control levels ($p < 0.05$) after 7 days of drug exposure, rising to a value not significantly different from control by 14 days of continuous drug exposure.

Erythroid cells in the older mouse spleens were more numerous in the presence of indomethacin (table 1), increasing 4–8-fold by 21 days of treatment when compared with control (day 0). Myeloid cells in the older mouse spleens also increased progressively in absolute numbers by 2–4 times control levels with increase in drug



Immature erythroid, myeloid and lymphoid cells in the spleen (a) and bone marrow (b) of young adult (7–9 weeks) mice. Tritiated (^{3}H-)thymidine-labelled cells, analyzed 1 h after isotope injection, are represented as a proportion (%) of all the morphologically immature cells within each lineage. Mean ± SE: 5 mice. The asterisk indicates statistically significant differences from control (day 0).

exposure time. Lymphoid cells in the spleens of the older mice rose slightly, but progressively, to levels 15% and 24% higher than control (day 0) levels, by 6–7 days and 21 days of treatment, respectively (table 1). Cells of the monocyte-macrophage lineage in the older mouse spleens increased only slightly in the presence of the drug. In the bone marrow of the older mouse, the size of the erythroid population was virtually unchanged during indomethacin treatment (table 1). Myeloid cells in the older mouse bone marrow, however, became more numerous during drug exposure (although the increase was much less pronounced than that of the corresponding spleen) being elevated by 45% and 23% over control at day 6–7 and 21, respectively of treatment (table 1). Lymphoid cells in such bone marrow fell to approximately 1/3 of their control (day 0) numbers by 21 days of indomethacin exposure, while cells of the monocyte-macrophage lineage remained unaffected throughout drug treatment. In neither the spleen nor the bone marrow of the older mouse did the relative numbers of immature vs mature cells within the lymphoid cell lineage change in the presence of the drug (table 2). In both the spleen and the bone marrow of the untreated, normal older mouse, the large majority of cells within the myeloid lineage are immature precursors (table 2). The ratio of immature to mature cells in the myeloid lineage remained unchanged throughout indomethacin exposure in both organs (table 2).

Discussion

Although extramedullary erythropoiesis appears much lower in older mice than in young mice, erythroid precursors in the spleen in both age groups nevertheless, proliferated intensely, as evidenced by the manifold increases at both ages in splenic erythroid cells, in the presence of indomethacin. No clinical evidence of hemorrhage, the dose-dependent side effect of indomethacin and its related compounds, i.e., acetylsalicylic acid, was observed, although in vivo these drugs can produce splenomegaly[11], megakaryocyte/platelet disfunction and prolonged bleeding time[25–27], events relating to chronic hemorrhage. In the present situation, bone marrow erythropoiesis, in both age groups, was not only less severely influenced than that of the extramedullary site (spleen), but normalization of erythroid cell numbers, in spite of continued drug presence, had already taken place at a time when it was still significantly above normal in the spleen. Such normalization may represent an adaptive response, mediated through one or more mechanisms, since it occurred during continued drug exposure. Increased demand for peripheral red cells (erythrocytes) during drug exposure may be met by the spleen, possibly allowing erythropoiesis in the bone marrow to return to normal levels. The previously demonstrated reduced capacity of the bone marrow stroma from normal, older mice to sustain erythropoiesis[28,29], may account in the present instance for the reduction in erythroid cell response in indomethacin-treated older mouse bone marrow relative to that of the young adult mouse. Regardless of mechanism, it appears that grossly undetectable red blood cell loss is taking place in the indomethacin-treated animal causing the observed stimulated (increased) erythropoiesis. In older mice, the early and progressive increase in splenic erythroid cell numbers with no corresponding change vs control in the bone marrow erythroid cell numbers throughout drug treatment indicates a capacity for erythroid compartment expansion in the spleen, but not the bone marrow which may already be maximally functional in the older animals.

In the young adult mouse, indomethacin raised the numbers of erythroid cells engaged in DNA synthesis in the spleen only. The drug may, in these younger mice, stimulate erythropoiesis, some cells for which may have been exported by the space-limited bone marrow compartments to become resident in the spleen, giving rise to the significant increase in both erythroid cells numbers and proportions of such precursors in DNA synthesis in the spleen.

The slight increase in the myeloid population in the spleens of the young adult mouse was early and transient and primarily reflected an increase in the numbers of immature cells in that lineage. Simultaneously, an equally early and transient decrease in bone marrow myeloid cells occurred. The inversion in relative numbers of immature to mature cells in the bone marrow and spleen at the early treatment intervals suggests some trafficking of immature cells from the bone marrow to the spleen. It has been found previously[20] that soon after indomethacin treatment, immature granulocyte precursors could be found in the blood concomitant with an increase in CFU-c in the bone marrow. The sensitivity of hemopoietic cells normally responding to the suppressive influences of PGE_2 increases in old age as does the endogenous level of PGE_2[17,30,31]. Inhibition of PGE_2 production by indomethacin would release cells from PGE_2-mediated suppression, accounting for the elevation in the numbers of myeloid cells in both the spleen and bone marrow of the older mouse. Unlike the young adult mouse, the relative proportions of immature and mature myeloid cells remained unchanged throughout drug treatment in the older mouse spleen and bone marrow, possibly refecting a reduced response to endogenous regulators of myeloid cell trafficking, but not proliferation, since the myeloid population size, as a whole, increased in both the spleen and the bone marrow during indomethacin exposure. Proportionately, the greater degree of stimulation of myelopoiesis in the older mouse spleen relative to the bone marrow, during indomethacin treatment, suggests that extramedullary myelopoiesis is more sensitive to the drug than is myelopoiesis centrally (bone marrow).

Negative influences of the drug in vivo and in vitro have been observed on proliferative responses of peripheral T and B lymphocytes[18,32,33], and would readily explain the present observation of reduced splenic lymphoid cell

numbers in the young adult mouse. In older mouse spleens, release of lymphoid cell proliferation, maturation and differentiation from the effects of PGE_2-enhanced T suppressor cells which are present in increaseing abundance in older mice[17,30] may explain the observed rise in splenic lymphoid cell numbers in older mice. Although increased export of lymphoid cells from the bone marrow to the spleen would account for a simultaneous decline and increase in the bone marrow and splenic lymphoid cells, respectively, in the older mouse, the numbers of such cells available for export from the bone marrow at each indomethacin treatment period would be too few to account for the increase in splenic lymphoid cell numbers at those intervals. Thus, augmented local proliferation during indomethacin treatment appears to account for the increase in splenic lymphoid cell numbers in the older mouse spleen.

The increase in monocyte-macrophage cell numbers in both the young adult and older mouse spleen is probably secondary to the inhibition of PGE_2 production by indomethacin, the former being inhibitory to monocyte-macrophage precursor proliferation[12,16,34].

Thus, the main hemopoietic organs, i.e., the bone marrow and the spleen are affected differentially during the course of indomethacin administration in vivo. The drug has a marked effect on every hemopoietic cell lineage in the spleen, irrespective of age. By contrast, in the bone marrow, the effect of the drug is not only less pronounced, but indomethacin-disturbed cell populations normalize fairly promptly, even during continued exposure to the drug. The bone marrow, consequently, may be a relatively protected organ, as the ultimate source of all hemopoietic and immune cell lineages, from the potentially severe effects of this drug, and possibly others.

Acknowledgments. This work was financially supported by a grant from the Cancer Research Society, Inc. The assistance of F. Leonard Christopher and Isabelle Dussault in performing the experiments with older mice is greatly appreciated.

1 Goodwin, J. S., J. Immunopharm. *2* (1980) 397.
2 Fulton, A. M., Cancer Res. *44* (1984) 2416.
3 Parhar, R. S., and Lala, P. K., J. exp. Med. *165* (1987) 14.
4 Lala, P. K., Parhar, R. S., Mertens, W., McFarlane, D., and Banergee, D., Proc. AACR *30* (1989) 379.
5 Vane, J. R., Nature, New Biol. *223* (1971) 231.
6 Lala, P. K., Parhar, R. S., and Singh, P., Cell. Immun. *99* (1986) 108.
7 Williams, N., Blood *53* (1979) 1089.
8 Brunda, M. J., Herberman, R. B., and Holden, H. T., J. Immun. *124* (1980) 2682.
9 Rogers, T. J., Dehaven, J. I., Donnelly, R. P., and Lamb, B., Cell. Immun. *87* (1984) 703.
10 Chouiab, S., Welte, K., Mertelsmann, R., and Dupont, B., J. Immun. *135* (1985) 1172.
11 Nikcevich, D. A., Young, M. R., Ellis, N. K., Newby, M., and Wepsic, H. T., J. Immunopharm. *8*(3) (1986) 299.
12 Gentile, P. S., and Pelus, L. M., Exp. Hemat. *15* (1987) 119.
13 Kohl, S., Jansen, D. M., and Loo, L.-S., Prost. Leuk. Med. *9* (1982) 159.
14 Pedersen, B. K., Oxholm, P., and Klarlund, K., Allergy *41* (1986) 532.
15 Voth, R., Chmielarczyk, W., Storch, E., and Kirchner, H., Nat. Immun. Cell Growth Reg. *5* (1986) 317.
16 Nilsson, B., Olofsson, T., and Olsson, I., Exp. Hemat. *12* (1984) 91.
17 Licastro, F., and Walford, R. L., Gerontology *32* (1986) 1.
18 Seng, G. F., Gay, B. J., and Bayer, B. M., Biochem. Pharmac. *36* (1987) 507.
19 Mannie, M. D., Pope, L., and Paterson, P. Y., Cell. Immun. *121* (1989) 196.
20 Fontagne, J., Adolphe, M., Semichon, M., Zizine, L., and Lechat, P., Exp. Hemat. *8* (1980) 1157.
21 Miller, S. C., and Osmond, D. G., Exp. Hemat. *2* (1974) 227.
22 Miller, S. C., and Osmond, D. G., Cell & Tissue Kinet. *8* (1975) 97.
23 Jacobsen, K., Tepper, J., and Osmond, D. G., Exp. Hemat. *18* (1990) 304.
24 Yoshida, Y., and Osmond, D. G., Blood *37* (1971) 73.
25 Minsker, D. H., and Kling, P. J., Thromb. Res. *10* (1977) 619.
26 Demers, L. M., Budin, R. E., and Shaikh, B., Proc. Soc. exp. Biol. Med. *183* (1980) 24.
27 Sullivan, P. B., and McDonald, T. P., Proc. Soc. exp. Biol. Med. *194* (1990) 216.
28 Hotta, T., Hirabayashi, N., Utsumi, M., Murate, T., and Yamada, H., Exp. Hemat. *8* (1980) 933.
29 Wolf, N. S., and Arora, R. K., Mech. Aging Dev. *20* (1982) 127.
30 Goodwin, J. S., and Messner, R. P., J. clin. Ivest. *64* (1979) 434.
31 Rosenstein, M. M., and Strausser, H. L., J. Reticuloend. Soc. *27* (1980) 159.
32 Panush, R. S., and Anthony, C. R., Clin. exp. Immun. *23* (1976) 114.
33 Rojo, J. M., Barasoain, I., and Portoles, A., Int. J. clin. Pharm. Ther. Toxic. *19* (1981) 220.
34 Kurland, J. I., Broxmeyer, H. E., Pelus, L. M., Bockman, R. G., and Moore, M. A. S., Blood *52* (1978) 388.

0014-4754/92/070674-05$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1992