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EFFECT OF AGE ON LEUKOTRIENE B_4 PRODUCTION IN GUINEA PIG WHOLE BLOOD

STEPHEN M. SPAETHE,* COURTNEY L. SCAIFE,† PENELOPE A. PECHOUS and ELDWIN L. VANALSTYNE

Department of Pulmonary Research, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285, U.S.A.

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Abstract—We evaluated the effect of age on eicosanoid production in guinea pig blood. Heparinized blood from 7–10-day, 6-week, or 6-month-old guinea pigs was incubated with 150 μM arachidonic acid (AA) for 5 min, followed by stimulation with A23187 (20 μg/mL) for an additional 10 min at 37°. The reaction was terminated by centrifugation, and the production of plasma leukotriene (LT) B_4 and C_4, prostaglandin E_2 (PGE_2), and thromboxane B_2 (TXB_2) was determined by enzyme-linked immunoassay (ELISA). LTC_4, PGE_2, and TXB_2 formation were unaffected by age. In marked contrast, production of LTB_4 was increased 4- to 5-fold as age increased from 7–10 days (9.51 ± 2.07 ng/mL) or 6 weeks (8.83 ± 1.81 ng/mL) to 6 months (40.57 ± 9.66 ng/mL). To determine the effect of age on the total eicosanoid product profile, blood was stimulated in the presence of [^{14}C]AA, and plasma metabolites were separated by reverse-phase high pressure liquid chromatography (RP-HPLC) and quantitated using on-line radiochemical detection. In addition to increased LTB_4 production, a modest increase in 12-hydroxyeicosatetraenoic acid (12-HETE) production was also observed in the 6-month-old animals. Previous studies have demonstrated interference of 12-HETE in the immunoassay of LTB_4. Therefore, to validate the authenticity of the plasma leukotriene ELISA measurements, samples were precipitated with methanol and fractionated by RP-HPLC. The fractions co-eluting with [^{3}H]LTB_4 or [^{3}H]LTC_4 were dried under vacuum and reconstituted in ELISA buffer, and leukotrienes were quantitated. As seen previously, following HPLC purification LTB_4 production remained significantly elevated in the 6-month-old guinea pigs, whereas LTC_4 production was unaffected by age. To further document the selectivity of this effect on LTB_4 production, we evaluated the effect of increasing age on cyclooxygenase or phospholipase A_2 (PLA_2) activity. Neither cyclooxygenase nor PLA_2 activity was elevated as animals matured. In conclusion, the capacity of whole blood to produce LTB_4, but not LTC_4, TXB_2, or PGE_2, was elevated markedly in older animals.

Elucidation of the role of leukotrienes (LTs‡) in asthma, allergic diseases, and other inflammatory disease processes has been an area of intense research in recent years. Biological activities of the various 5-lipoxygenase products of arachidonate are diverse. Leukotrienes are potent airway smooth muscle constrictors (LTC_4, LTD_4, and LTE_4), act as pulmonary mucus secretagogues (LTC_4 and possibly LTB_4), and stimulate plasma exudation and airway edema (LTB_4 and LTC_4), activities all of which are manifested in human bronchial asthma. The leukotrienes also possess a broad range of pro-inflammatory properties. LTB_4, a potent leukocyte chemoattractant, stimulates intravascular leukocyte/endothelial cell adhesion via up-regulation of the leukocyte adhesion complex, CD11b/CD18, and promotes subsequent diapedesis out of the vascular space into surrounding tissues [1, 2]. Therefore, the development of a selective 5-lipoxygenase inhibitor has valuable therapeutic potential for ameliorating some of the symptoms of asthma and other inflammatory disease processes.

Quantitation of eicosanoids in plasma following the *ex vivo* stimulation of whole blood has proven to be a simple, yet effective method for evaluating the pharmacodynamic properties of cyclooxygenase and 5-lipoxygenase inhibitors *in vivo* in rabbits [3], mice [4], and rats [5]. We recently developed a whole blood 5-lipoxygenase assay in guinea pigs to evaluate the selectivity and pharmacodynamic properties of 5-lipoxygenase inhibitors *in vivo* [6]. In the course of assay development, we noted a high degree of animal-to-animal variability in LTB_4 production in stimulated whole blood. Previous studies in guinea pigs have demonstrated that age can affect dramatically the production of 5-lipoxygenase metabolites of arachidonic acid [7]. In the present report, we examine the effect of age on eicosanoid production in guinea pig blood.

MATERIALS AND METHODS

Whole blood eicosanoid production. Male Hartley

* Corresponding author: Stephen M. Spaethe, Ph.D., Department of Pulmonary Research, Eli Lilly & Co., Lilly Corporate Center, Indianapolis, IN 46285. Tel: (317) 276-7938; FAX (317) 276-1417.

† Courtney L. Scaife was a Howard Hughes Medical Foundation Undergraduate Fellow, DePauw University, Greencastle, IN 46135.

‡ Abbreviations: AA, arachidonic acid; BSA, bovine serum albumin; ELISA, enzyme-linked immunoassay; FMLP, *n*-formyl-Met-Leu-Phe; 12-HETE, 12-hydroxy-eicosatetraenoic acid; LT, leukotriene; PCA, passive cutaneous anaphylaxis; PGE_2, prostaglandin E_2; PLA_2, phospholipase A_2; RBC, red blood cell; RP-HPLC, reverse phase high pressure liquid chromatography; and TXB_2, thromboxane B_2.

guinea pigs (Charles Rivers, Portage, MI) of known age and weight were anesthetized with a combination of ketamine (35 mg/kg; Aveco Co., Fort Dodge, IA) and xylazine (10 mg/kg; Butler Co., Columbus, OH) administered subcutaneously. Heparinized blood (20 U/mL) was obtained via cardiac puncture and the whole blood *ex vivo* assay initiated within 5 min. Replicate 1-mL aliquots of blood were incubated with 150 μM sodium arachidonate (Nu Chek Prep, Elysian, MN) for 5 min at 37°, and stimulated by the addition of 20 μg/mL A23187 (supplied by Ms. Louise Crandall, Eli Lilly & Co.). The reaction was allowed to proceed for an additional 10 min at 37°, and was terminated by centrifugation at 4°. Plasma was stored at −32° prior to direct quantitation of eicosanoids. Previous studies have shown that co-stimulation with arachidonic acid (AA) and A23187 is required by leukotriene production by whole blood of guinea pigs. Additionally, the time course for leukotriene production was shown to be linear utilizing this experimental protocol [6].

Quantitation of eicosanoids by enzyme-linked immunoassay (ELISA). Eicosanoid ELISA assays were performed utilizing reagents obtained from the Cayman Chemical Co. (Ann Arbor, MI) as outlined in the instructions obtained therein.

RP-HPLC separation of AA metabolites and quantitation by ELISA. Studies have reported that production of 12-hydroxyeicosatetraenoic acid (12-HETE) in similar whole blood assay systems can interfere with quantitation of LTB_4 by immunoassay [8]. Consequently, a reverse phase high pressure liquid chromatography (RP-HPLC) method was developed which allowed for chromatographic fractionation of LTB_4, 12-HETE, and LTC_4. Blood was collected and stimulated as described above. Plasma was spiked with trace amounts of tritiated LTB_4, 12(*S*)-HETE, and LTC_4 (New England Nuclear, Boston, MA) to monitor recovery, deproteinated by the addition of methanol [60% (v/v) final concentration], and stored at −32°. Following centrifugation at 4°, the plasma/methanol extract was fractionated utilizing RP-HPLC.

A customized Hewlett–Packard 1090 HPLC system (Hewlett–Packard, Boblingen, Germany) was designed which allowed for consecutive automated, pre-column extractions of eicosanoids on a guard column prior to separation and fractionation by RP-HPLC*. Briefly, with the aid of a refrigerated autoinjector, an aliquot of methanolic plasma extract was applied to a C18 (7 μm, 0.32 × 1.5 cm i.d.) guard column (Brownlee Laboratories, Inc., Santa Clara, CA) and AA metabolites were eluted onto an Ultrasphere C18 (5 μm, 0.46 × 25 cm i.d.) analytical column (Beckman Instruments, Fullerton, CA). The mobile phase consisted of an acidic water (pH 3.3 with phosphoric acid)/methanol buffer containing 1.5 mM EDTA. Eicosanoids were separated utilizing a discontinuous methanol gradient [60–95% (v/v) over 22 min] at a flow rate of 1.8 mL/min. The fractions containing LTB_4 or LTC_4 were collected, dried under vacuum, reconstituted in ELISA buffer, and stored at −32° prior to quantitation by ELISA. With the aid of a pneumatic column switching apparatus coupled to an external solvent delivery system, cleaning and regeneration of the guard column were achieved automatically during HPLC separation. Recovery was 83.0 ± 1.0 and 90.4 ± 1.2% for [^{3}H]LTB_4 and [^{3}H]LTC_4, respectively.

Metabolic profile of [^{14}C]arachidonic acid in guinea pig blood. Heparinized blood was obtained via cardiac puncture as described. Aliquots (1 mL) of blood were incubated for 5 min at 37° with 150 μM [^{14}C]arachidonic acid (1.1×10^{-3} μCi/μmol final concentration; New England Nuclear). The reaction was further stimulated with 20 μg/mL A23187 for an additional 10 min at 37°, and terminated by centrifugation at 4°. Plasma was spiked with trace amounts of tritiated LTB_4 and 12(*S*)-HETE to monitor retention times, and deproteinated by the addition of methanol (final concentration = 55%). [^{14}C]AA metabolites were separated by RP-HPLC modified from Henke *et al.* [9] over an Ultrasphere C18 (5 μm, 0.46 × 25 cm i.d.) analytical column (Beckman Instruments). The mobile phase consisted of acidified water (pH 6.2 with acetic acid)/methanol containing 1.5 mM EDTA. Separation was achieved utilizing a discontinuous methanol gradient [55–100% (v/v) over 73 min] at a flow rate of 1.0 mL/min. Metabolities were detected using a Raytest-Ramona-5 liquid scintillation flow through detector (Raytest USA, Pittsburgh, PA) and quantitated by integration of peak area. Absolute amounts of plasma 12-HETE and AA were calculated by converting peak integration values (expressed as dpm) to concentration (μg/mL) based upon the molecular weight, and the initial specific activity of [^{14}C]AA.

Assessment of whole blood cyclooxygenase or phospholipase A_2 (PLA_2) activity. Cyclooxygenase activity is the rate-limiting enzymatic step in the conversion of arachidonate to prostaglandins and thromboxane [10]. Therefore, the formation of TXB_2 and PGE_2 following the stimulation of whole blood with AA alone is a direct assessment of cyclooxygenase activity. Likewise, the control of TXB_2 and PGE_2 production following A23187 stimulation is dependent upon the release of AA by PLA_2 [11]. In the absence of exogenous AA, A23187-stimulated TXB_2 and PGE_2 production is an indirect assessment of PLA_2 activity. The whole blood assays described thus far utilized co-stimulation protocols which were optimized for leukotriene production. Therefore, it was necessary to independently assess cyclooxygenase or PLA_2 activity under conditions of known linearity. To determine the activities of cyclooxygenase and phospholipase A_2 in whole blood, we modified the *ex vivo* assay as follows: heparinized whole blood was stimulated with either AA (150 μM) or A23187 (20 μg/mL) for 5 min at 37° to monitor cyclooxygenase or PLA_2 activity, respectively. The reaction was terminated by centrifugation and eicosanoids were quantitated as described. Eicosanoid production was linear over 5 min under these conditions (Spaethe SM and VanAlstyne EL, unpublished observations).

* VanAlstyne EL and Spaethe SM, *J Chromatogr*, manuscript submitted for publication.

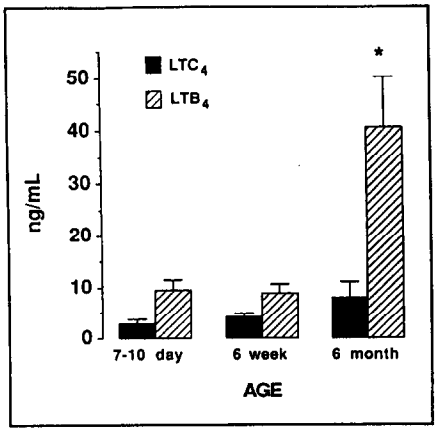

Fig. 1. Effect of age on leukotriene production in guinea pig blood. Heparinized whole blood was incubated with 150 μM sodium archidonate for 5 min at 37° prior to stimulation with 20 μg/mL A23187. The reaction was allowed to proceed for an additional 10 min and then was terminated by centrifugation. Plasma leukotriene concentrations were determined by ELISA (Values are means ± SEM, N = 5). Key: (*) significantly greater than either 7–10-day-old or 6-week-old animals, $P < 0.05$.

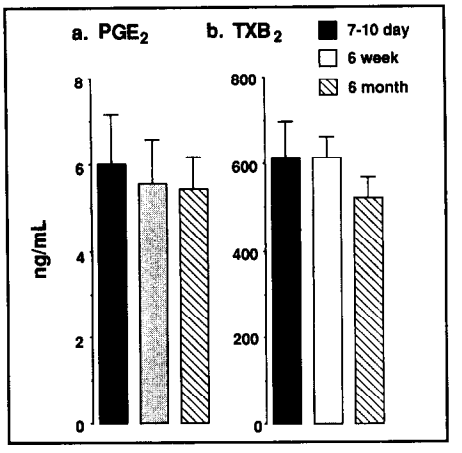

Fig. 2. Effect of age on PGE_2 (panel a) or TXB_2 (panel b) production in guinea pig blood. Heparinized whole blood was incubated with 150 μM sodium arachidonate for 5 min at 37° prior to stimulation with 20 μg/mL A23187. The reaction was allowed to proceed for an additional 10 min and was terminated by centrifugation. Plasma PGE_2 and TXB_2 concentrations were determined by ELISA. Values are means ± SEM, N = 5.

Determination of ciculating platelet, leukocyte, and red blood cell (RBC) counts. Total platelet, leukocyte, and RBC counts were determined using a Cell-Dyne 900 hematology analyzer (Sequoia–Turner Corp., Mountain View, CA).

Statistical analysis. Statistical analysis was performed utilizing one-way analysis of variance coupled with Scheffe's statistic for comparisons within groups [12]. Significant difference was determined at the level of $P < 0.05$.

RESULTS

Age-related increase in whole blood LTB_4 production. The effect of age on whole blood leukotriene production in response to co-stimulation with AA and A23187 was examined. Stimulation of whole blood resulted in a modest (2- to 4-fold) increase in LTC_4 above basal plasma immunoreactivity which was not affected significantly by age of the animal (7–10 day, 2.93 ± 0.76; 6 week, 4.12 ± 0.84; 6 month, 8.04 ± 3.03 ng/mL; Fig. 1). In marked contrast, production of LTB_4 was increased markedly in blood from 6-month-old animals (40.57 ± 9.66 ng/mL) as compared with 7–10-day-old (9.51 ± 2.07 ng/mL) or 6-week-old animals (8.83 ± 1.81 ng/mL) (Fig. 1). Production of the cyclooxygenase metabolites, PGE_2 (7–10 day, 6.00 ± 1.15; 6 week, 5.55 ± 1.03; 6 month, 5.43 ± 0.75 ng/mL) or TXB_2 (7–10 day, 615.20 ± 80.84; 6 week, 612.83 ± 47.50; 6 month, 520.5 ± 47.39 ng/mL), was not affected by age (Fig. 2, a and b).

Carey *et al.* [8] utilizing a similar whole blood assay system reported that cross-reactivity with the 12-lipoxygenase metabolite, 12-HETE, results in an over-estimation of immunoreactive plasma LTB_4 formation. To eliminate this potential interference of blood constituents in the immunoassays employed in these studies, we extracted and fractionated plasma leukotrienes by RP-HPLC prior to quantitation with ELISA. Quantitation of LTB_4 following chromatographic purification demonstrated that 6-month-old guinea pigs indeed produced 4- to 5-fold more immunoreactive LTB_4 than the younger animals (7–10 day, 38.67 ± 6.59; 6 week, 36.04 ± 7.47; 6 month, 163.67 ± 24.2 ng/mL; Fig. 3). In marked contrast, the modest production of the cysteinyl-containing leukotriene, LTC_4, was not altered with age (7–10 day, 4.25 ± 0.93; 6 week, 4.33 ± 0.92; 6 month, 5.75 ± 0.24 ng/mL; Fig. 3).

Effect of age on [^{14}C]arachidonate metabolism in whole blood. The age-related increase in plasma LTB_4 observed following stimulation of whole blood with AA/A23187 could either be explained by increased synthesis of this metabolite or it could be a result of decreased degradation of LTB_4. To determine the effect of age on the profile of arachidonate metabolism, blood from guinea pigs of various ages was incubated in the presence of [^{14}C]-arachidonate prior to stimulation with A23187. Plasma [^{14}C]AA metabolites were separated and quantitated. Significant accumulation of the ω-hydroxylation products of LTB_4, 20-OH-LTB_4 and 20-COOH-LTB_4, was not observed in any age group tested. The major [^{14}C]metabolite co-eluted with tritiated 12(*S*)-HETE as previously reported for rats and mice [8]. Production of this metabolite was also elevated in 6-month-old guinea pigs (1.81 ± 0.27 μg/mL) when compared to 7–10-day-old (1.16 ± 0.09 μg/mL) or 6-week-old (1.12 ± 0.10 μg/mL) animals (Fig. 4). While chiral analysis was not performed to determine the stereoisomeric assignment of this metabolite, it is likely that the 12-HETE found in plasma is a product of the platelet

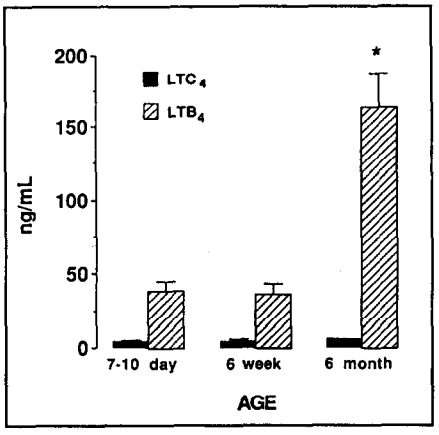

Fig. 3. Effect of selective RP-HPLC fractionation on immunoreactive LTC_4 and LTB_4 production in guinea pig blood. Heparinized whole blood was incubated with 150 μM sodium arachidonate for 5 min at 37° prior to stimulation with 20 μg/mL A23187. The reaction was allowed to proceed for an additional 10 min and was terminated by centrifugation. Plasma proteins were precipitated and the specific LTB_4 fraction was collected and quantitated as described under Materials and Methods. Values are means ± SEM, N = 5. Key: (*) significantly greater than either 7–10-day-old or 6-week-old animals, $P < 0.05$.

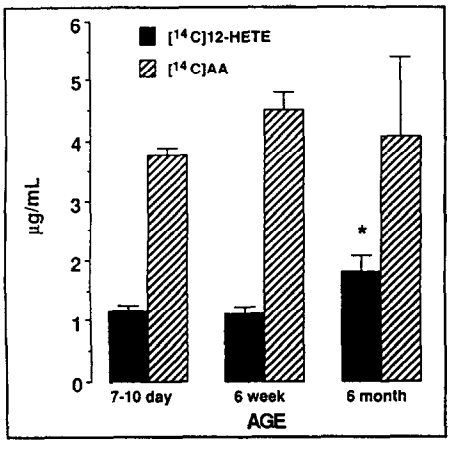

Fig. 4. Effect of age on [^{14}C]AA metabolism in guinea pig blood. Heparinized whole blood was incubated with 150 μM [^{14}C]AA (1.1×10^{-3} μC/mL) for 5 min at 37° prior to stimulation with 20 μg/mL A23187. The reaction was alllowed to proceed for an additional 10 min and was terminated by centrifugation. Metabolites were monitored by on-line flow scintillation detection and quantitated by integration of peak area. Absolute amounts of plasma 12-HETE and AA were calculated as described under Materials and Methods. Values are means ± SEM, N = 5. Key: (*) significantly greater than either 7–10-day-old or 6-week-old animals, $P < 0.05$.

12-lipoxygenase [8], and therefore the 12(*S*) stereoisomer [13]. Free plasma arachidonate was not altered with age (Fig. 4).

Effect of age on cyclooxygenase or PLA_2 activity.

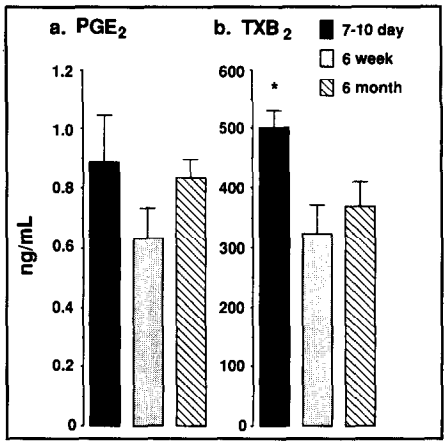

Fig. 5. Effect of age on cyclooxygenase activity in guinea pig blood. Heparinized whole blood was stimulated with 150 μM sodium arachidonate for 5 min at 37° and terminated by centrifugation. Plasma PGE_2 (panel a) and TXB_2 (panel b) concentrations were determined by ELISA. Values are means ± SEM, N = 5. Key: (*) significantly greater than either 6-week- or 6-month-old animals, $P < 0.05$.

Cyclooxygenase activity as determined by PGE_2 production was not altered as animals matured from 7–10 days (888 ± 160 pg/mL) to 6 weeks (632 ± 99 pg/mL) or 6 months of age (832 ± 66 pg/mL) (Fig. 5a). Thromboxane production, however, was modestly (approx. 30%), though significantly lower in the 6-week-old (323 ± 47 ng/mL) or 6-month-old guinea pigs (368 ± 41 ng/mL) compared with 7–10-day-old animals (501 ± 28 ng/mL) (Fig. 5b).

As an indirect assessment of PLA_2, whole blood was stimulated with A23187. PLA_2-dependent TXB_2 and PGE_2 production was not increased with increasing age (7–10-day-old, PGE_2, 3.47 ± 0.33 ng/mL, TXB_2, 246 ± 6 ng/mL; 6-week-old, PGE_2, 4.15 ± 0.57 ng/mL, TXB_2, 232 ± 11 ng/mL; and 6-month-old, PGE_2, 3.56 ± 0.45 ng/mL, TXB_2, 247 ± 8 ng/mL; Fig. 6, a and b).

Effect of age on platelet, leukocyte and red blood cell counts. A possible explanation for differences in the production of these metabolites could be due to variations in cell numbers as a function of age. Neither platelet nor total leukocyte count was altered in the 6-month-old animals as compared with either the 7–10 day-old or 6-week-old guinea pigs. However, there was a small, though significant increase in the red blood cell count with increasing age. Red blood cells are known to contribute to the production of LTB_4 in whole blood [14, 15]. Six-month-old animals had 27 and 23% greater circulating red blood cells than the 7–10-day-old or 6-week-old guinea pigs, respectively (Table 1), which may account for some of the differences observed. When the data were normalized for red blood cell numbers, a 3- to 4-fold increase in whole blood LTB_4 production in the older animals was observed in plasma (7–10 day, 2.23 ± 0.48; 6 week, 2.00 ± 0.41; 6 month, 7.46 ± 1.78 ng/10^9 RBC) or following HPLC purification (7–10 day, 9.06 ± 1.54; 6 week, 8.77 ± 1.69;

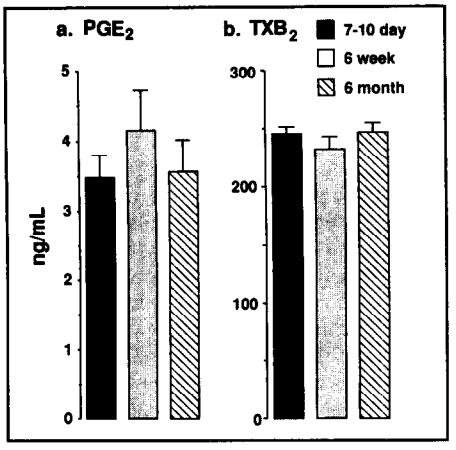

Fig. 6. Effect of age on PLA_2 activity in guinea pig blood. Heparinized whole blood was stimulated with 20 μg/mL A23187 for 5 min at 37° and terminated by centrifugation. Plasma PGE_2 (panel a) and TXB_2 (panel b) concentrations were determined by ELISA. Values are means ± SEM, N = 5.

6 month, 30.09 ± 4.45 ng/10^9 RBC). This would indicate that a minor component of the age-related increase in whole blood LTB_4 may well be due to a modest increase in red blood cell number in older animals.

DISCUSSION

In the present report, an age-dependent increase in the production of LTB_4 in guinea pig blood was observed. This increase was specific for the LTA hydrolase-dependent 5-lipoxygenase product, LTB_4, in that there was no concurrent increase in the production of the cysteinyl-containing leukotriene, LTC_4, or the cyclooxygenase metabolites, PGE_2 or TXB_2. However, assay conditions were optimized for leukotriene production, so further assays to directly assess cyclooxygenase or PLA_2 activity were performed. Incubation of guinea pig whole blood with sodium arachidonate in the absence of A23187 (i.e. as a direct measure of cyclooxygenase activity) resulted in the production of TXB_2 and PGE_2, but not LTB_4 (or LTC_4). Neither TXB_2 nor PGE_2 was increased with increasing age. A modest decrease in TXB_2 production was noted as animals matured from 7–10 days old to 6 weeks old. The significance of this was not explored. A similar modest decrease in TXB_2 production in whole blood of rats in response to thrombin has been reported [16]. Takahashi *et al.* hypothesized that this age-dependent reduction in TXB_2 production was related to plasma cholesterol levels. A similar mechanism may be responsible for this observation in the present report.

We next assessed the effect of increasing age on PLA_2 activity. Unlike other species [3–5, 17], stimulation of guinea pig whole blood with A23187 alone resulted in the production of the cyclooxygenase metabolites PGE_2 and TXB_2 without an accompanying increase in LTB_4 (or LTC_4). A23187-stimulated PGE_2 and TXB_2 production, an indirect assessment of PLA_2 activity, was not altered with increasing age.

The physiological or pathological significance of these observations is, at present, unknown. LTB_4 is a potent mediator of acute inflammation. This 5-lipoxygenase metabolite stimulates a variety of pro-inflammatory leukocyte responses including aggregation, adhesion, chemokinesis, chemotaxis, and degranulation *in vitro* and *in vivo*, and diapedesis and plasma extravastion *in vivo* [1, 2]. Leukotriene B_4 is synthesized by leukocytes [10] and, therefore, LTB_4 potentially acts as an endogenous amplifier of leukocyte-mediated tissue injury in acute (and chronic) inflammation.

Very few studies on the ontogeny of the acute inflammatory response have appeared in the literature. *In vitro* studies have reported that neutrophils isolated from human neonates have a lower chemotactic responsiveness when compared to cells isolated from the adult [18]. *In vivo* studies by Schuit and Homisch [19] in rats have corroborated these findings. Intraperitoneal administration of streptococcus, or the *n*-formylated oligopeptide, *n*-formyl-Met-Leu-Phe (FMLP) resulted in a dramatic increase in neutrophil influx into the peritoneal cavity in adult rats. In marked contrast, the neutrophil influx in response to either of these inflammatory stimuli was completely absent in neonatal rats. Schuit and Homisch hypothesized that the decreased inflammatory cell influx in the neonates

Table 1. Effects of age on weight, and platelet, leukocyte, and red blood cell count in the guinea pig

Age	Weight* (g)	Platelets† (×10^6/mL)	Leukocytes* (×10^6/mL)	RBCs† (×10^9/mL)
7–10 days	182 ± 6‡§	317.6 ± 56.8	6.30 ± 0.70	4.27 ± 0.17§
1–2 months	372 ± 6§‖	278.6 ± 9.2	5.35 ± 1.61	4.41 ± 0.09§
6 months	927 ± 38‡‖	266.2 ± 44.61	4.22 ± 0.39	5.44 ± 0.19

* Values are means ± SEM, N = 10.
† Values are means ± SEM, N = 5.
‡ Significantly different from 2-month-old, $P < 0.05$.
§ Significantly different from 6-month-old, $P < 0.05$.
‖ Significantly different from 7–10-day-old, $P < 0.05$.

was due to an intrinsic defect in chemotactic responsiveness, possibly as a result of plasma membrane and cytoskeletal abnormalities. A second hypothesis could be implicated from the results reported in the present study. The inflammatory cell agonist, FMLP, stimulates LTB_4 production in neutrophils [20] and in peritoneal leukocytes [21]. Therefore, the absence of inflammatory cell influx observed in neonatal rats could be a result of decreased production of LTB_4 in response to FMLP.

Other investigators have observed similar effects of age on the acute inflammatory response in experimental animal models. In a model of passive cutaneous anaphylaxis (PCA) in rabbits passively sensitized to ovalbumin, adult animals respond to antigen challenge with the characteristic increase in vascular permeability and neutrophil infiltrate. Antigen challenge of newborn animals, however, resulted in virtually no PCA; no increase in vascular permeability or influx of inflammatory cells was observed [22]. Similar observations were observed in rabbits actively sensitized with bovine serum albumin (BSA). Administration of BSA in the presence of aluminum hydroxide to newborn rabbits resulted in the long-term synthesis of IgE anti-BSA associated with the development of systemic anaphylaxis at 3 months of age [23]. In contrast, systemic anaphylaxis was absent at 4–6 months of age, even though these animals had maximal circulating levels of anti-BSA IgE [22]. Leukotrienes are known mediators of certain of the pathophysiological events which occur during systemic anaphylaxis (i.e. bronchoconstriction, plasma exudation and edema, and leukocyte activation and extravasation). Meng *et al.* reported that, in adult animals, systemic anaphylaxis was apparent within 2 min of antigen administration and was characterized by respiratory distress and death due to "hypoxia" [22], pathophysiological effects often attributed to leukotrienes [1, 2]. Therefore, decreased leukotriene production may have had a role in the abrogated response to antigen challenge in the young animals. However, a report by Fleisch *et al.* [7] and the findings reported here indicate that younger animals produce either equivalent or greater amounts of cysteinyl-containing leukotrienes. The biological properties of this series of 5-lipoxygenase metabolites are the properties associated most often with the pathogenesis of systemic anaphylaxis [1, 2]. However, the role of LTB_4 in pulmonary changes which occur during systemic anaphylaxis is less well defined, and future studies are needed to elucidate the role of this 5-lipoxygenase metabolite as well as the effect of age on the pathogenesis of hypersensitivity reactions.

In conclusion, in contrast to the age-related decrease in antigen-induced mediator release from guinea pig chopped lung [7], there was an age-dependent increase in LTB_4 production in guinea pig whole blood, which was not a result of changes in circulating leukocyte number. This was a real increase in the production of LTB_4, not a result of diminished catabolism of LTB_4 or interference in the immunoassay of LTB_4 by 12-HETE. Unlike the cyclooxygenase system where cyclooxygenase itself is the rate-limiting enzyme in the production of TXB_2 and PGE_2 following the release of AA by PLA_2, the production of LTB_4 is not limited by the activity of 5-lipoxygenase, but rather by LTA hydrolase activity [10]. Additionally, we did not observe an age-related increase in the production of the cysteinyl-containing, LTA hydrolase-independent leukotriene, LTC_4. Therefore, an increase in (1) the amount of, or (2) the activity of LTA hydrolase in older animals could also explain these results. The minor increase in erythrocyte number noted with increasing age may contribute to the increase in LTB_4 production in whole blood since these cells represent a major source of LTA synthase [14, 15] and are the predominant cell type present. A third possible, though unprecedented, explanation for the effect of age on LTB_4 production is an increase in a "5-lipoxygenase/LTA hydrolase specific" PLA_2 activity. The mechanism of enhanced LTB_4 production and the pathophysiological significance of this observation are under investigation.

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