0031-3998/95/3801-0049$03.00/0
PEDIATRIC RESEARCH

Vol. 38, No. 1, 1995
Printed in U.S.A.

Secretion of Transforming Growth Factor-α (TGFα) by Postnatal Rabbit Alveolar Macrophages

CAROL L. WAGNER, RITA M. RYAN, DONNA E. FORSYTHE, ANDREA KEENAN, AND JACOB N. FINKELSTEIN

Department of Pediatrics, Division of Neonatology, Medical University of South Carolina, Charleston, South Carolina 29401 [C.L.W., D.E.F., A.K.] and Department of Pediatrics, Division of Neonatology, University of Rochester, Strong Children's Research Center, Rochester, New York 14642 [R.M.R., J.N.F.]

ABSTRACT

Transforming growth factor-α (TGFα) is a cytokine secreted by stimulated alveolar macrophages (AM) *in vitro* and after *in vivo* particulate or hyperoxia exposure and has been implicated in the processes of postnatal lung development. It is unknown if AM TGFα secretion changes during normal postnatal lung development. After sacrifice of New Zealand white rabbits on postnatal d 0–2, 5–7, 9–10, 14, 21, and 28 and >4 mo (adult), AM were isolated by discontinuous density centrifugation and placed in culture in the presence or absence of concanavalin A (ConA) for 24 h. Media were collected, and the concentration and isoforms of TGFα in AM media samples were determined by an epidermal growth factor/TGFα radioreceptor assay and Western immunodetection, respectively. TGFα was present in media of AM from the 1.06 and 1.08 g/dL Percoll densities, but not in the 1.10 g/dL density. Statistically significant differences in TGFα secretion by unstimulated and ConA-stimulated AM at the various ages were not detected until d 14 ($p < 0.02$). Western blot analysis of unstimulated AM media samples from d 0–7 rabbits demonstrated the presence of TGFα isoforms at 46, 30, and 14.3 kD. At later postnatal ages (≥ d 9), a single 14.3-kD isoform was present. In contrast, analysis of ConA-stimulated AM media samples showed TGFα isoforms at 46, 30, and 14.3 kD for all ages; however, the 6-kD mature isoform was present only in juvenile (d 28) and adult media. These results demonstrate an age-dependent effect on AM TGFα secretion and biochemical isoforms. (***Pediatr Res* 38: 49–54, 1995**)

Abbreviations

AM, alveolar macrophages
TGFα, transforming growth factor-α
EGF, epidermal growth factor
ConA, concanavalin A
DME, Dulbecco's modified Eagle's medium

The expression and secretion of TGFα by activated adult human AM were first demonstrated by Madtes *et al.* in 1988 (1). Subsequently, TGFα was identified in culture medium of AM derived from rabbits recovering from hyperoxic injury and after *in vitro* activation of AM from control rabbits (2–7). There is further evidence that TGFα stimulates type II cell proliferation (3, 4) and, therefore, may play a role in the maintenance and reparative processes of the lung.

After birth, the lung undergoes continued growth and differentiation of parenchyma and supporting tissue. The volume and surface area of the lung at birth is less than 10% of the total lung tissue present in the adult (8). Growth factors such as TGFα, many of which can be produced by AM, have been implicated in the perpetuation of normal lung growth and differentiation through their interaction with type II epithelial cells and extracellular matrix (9–13).

Through the prior work of several investigators (14–24), it is known that an influx of AM into the rabbit lung precedes birth by 24 h. This is followed by an exponential increase in AM number during the first week after delivery. During postnatal lung development, there is a gradual shift from a homogeneous, monocyte-like population to a more heterogeneous population of AM of varying density and function (14, 25–30). Although the ultrastructure and certain biologic functions of AM isolated from postnatal rabbits have been defined (14–25), there is scarce information about AM growth factor production and what effect such cytokines may have on lung parenchymal differentiation during postnatal development. It is unknown when AM become functionally capable of TGFα secretion during postnatal development and if this capability extends to one specific subpopulation of AM (28, 29).

Several isoforms of TGFα have been isolated in other systems, with molecular mass ranging from 6 to 20–22 kD

Received September 19, 1994; accepted February 9, 1995.
Correspondence and reprint requests: Carol L. Wagner, M.D., Medical University of South Carolina, Children's Hospital, 171 Ashley Avenue, Charleston, SC 29425.
Supported in part by National Institutes of Health Grant F32-HL08462.

(9–13). Activation of the EGF receptor by both transmembrane proforms of TGFα as well as the 6-kD secreted mature form has been shown. The processing of higher molecular mass proforms of TGFα to the 6-kD mature form is dependent on enzymatic activity that may not be fully developed at birth (9–13). Whether certain isoforms of TGFα secreted by AM predominate during postnatal development has yet to be determined.

These current experiments measured *in vitro* TGFα secretion by AM isolated from rabbits under conditions of normal lung growth. The hypothesis that AM isolated from younger postnatal rabbits would show differences in both cell density and TGFα secretion compared with adult AM was tested. It was further hypothesized that there would be an age-dependent difference in the TGFα isoforms secreted by AM.

METHODS

The postnatal secretion of TGFα by AM was studied in a rabbit model previously described (2–4). Animals were bred, born, and housed in a single room designated for New Zealand white rabbits in the Animal Care Facility at the University of Rochester. Litter mates were kept in a large cage with the doe and allowed to feed *ad libitum*. Each animal was evaluated daily by one of the investigators or animal laboratory personnel. Those animals who showed evidence of respiratory infection or gastrointestinal disease were sacrificed and excluded from study. All animals included for study were in good health at the time of sacrifice.

The rabbits were sacrificed at d 0–2, 5–7, 9–10, 14, 21, and 28, and at >4 mo (2–3-kg adult males). AM were isolated by bronchoalveolar lavage, separated from red blood cells and cellular debris by discontinuous Percoll density gradients (1.061, 1.08, and 1.10 g/dL), and centrifugation at 1000 × *g* for 20 min at 4°C. To obtain sufficient numbers of AM for bioassay, lavage samples from d 0–7 rabbits were pooled. Thereafter (≥ d 9–10), there were sufficient numbers of AM for experimental study of individual animals. After separation of AM by Percoll, each Percoll layer present was washed in Hank's buffered saline (Life Technologies, Inc, Grand Island, NY), 300 × *g* for 6 min at 4°C, and then resuspended at a concentration of 1×10^6 cells/mL of DME-F12 medium (Sigma Chemical Co., St. Louis, MO) with gentamicin sulfate (Sigma Chemical Co.). Viability of each subpopulation was determined using a trypan blue exclusion method (Sigma Chemical Co.). In all samples tested, there was a minimum of 95% viable cells. Confirmation of cell type was performed by Diff-Quik cell differential staining (Harleco, Philadelphia, PA) with >95% of the cells identified as mononuclear phagocytes at all ages tested.

AM were plated in culture at a concentration of 1×10^6 cells/mL DME-F12 medium alone or with ConA (Sigma Chemical Co.) at a concentration of 10 μg/mL for 24 h. After 24 h of incubation at 37°C in a humidified atmosphere of 5% CO_2/95% air, the media were removed from each Petri dish, centrifuged to remove nonadherent cells and particles, and concentrated 5-fold using Centricon-3 filters (3-kD molecular mass cutoff; Amicon, Beverly, MA).

EGF-like activity in the media samples was measured by an EGF/TGFα radioreceptor assay (Biomedical Technologies, Boston, MA) that utilizes competitive binding of ^{125}I-TGFα and media or recombinant 6-kD TGFα (Oncogene Science, Uniondale, NY) to A431 membranes. The sensitivity of the assay was 0.2 ng/mL. All samples were assayed in duplicate or triplicate. Inasmuch as AM do not produce or secrete EGF (10), competitive binding of conditioned media and ^{125}I-TGFα to A431 membranes, with known EGF receptors, reflected TGFα activity.

TGFα isoforms secreted by AM at the various postnatal ages were identified by Western blot analysis. A standard electrophoresis protocol was followed (31). Fifty microliters of each sample were loaded per lane onto a 4% stacking gel and electrophoresed through a 15% SDS-polyacrylamide gel. Prestained low molecular mass rainbow markers (range 2,350–46,000 kD; Amersham Corp., Arlington Heights, IL), human recombinant TGFα standard in acetate buffer (molecular mass 6 kD; 10 ng; Oncogene Science), and media samples were run in parallel lanes under reducing conditions (2-mercaptoethanol; Sigma Chemical Co.). Each 10-cm gel was run at room temperature over 3–4 h at 35 mA/gel.

After electrophoresis, polyacrylamide gels were washed in a Tris base cathode buffer for removal of SDS. Proteins, including standard molecular mass markers and reference TGFα standard, were transferred to nitrocellulose (0.45-μm pore; Amersham Corp.) using the semidry method (Millipore Graphite Electroblotter 1; Millipore, Bedford, MA) (32). Membranes were blocked with Tween Tris base solution (TTBS: 0.1% Tween-20, 0.15 M sodium chloride and 0.01 M Tris-HCl, pH 7.6) containing 5% nonfat dry milk in TTBS for 1 h at room temperature, then washed several times in TTBS, followed by 1-h blocking with 3% BSA (Sigma Chemical Co.).

The washed nitrocellulose then was incubated with a primary monoclonal mouse-anti-human TGFα antibody (4 μg/mL in TTBS; Research Diagnostics, Flanders, NJ) overnight on a rocker at 4°C. The membrane was washed and then incubated in a secondary goat-anti-mouse horseradish peroxidase-conjugated polyclonal antibody (Research Diagnostics) 1:3000 in TTBS. All Western blots were carried out with the necessary secondary antibody alone to detect nonspecific binding. Subsequently, each nitrocellulose membrane was washed and then treated with enhanced chemiluminescence specific for horseradish peroxidase coupled to biotin/avidin (Amersham Corp.). The membrane was placed with radiographic film for visualization of the molecular mass band(s) indicative of TGFα isoform(s).

Statistical analysis. The concentration of TGFα in each sample as determined by RIA is expressed as the mean ± SD of triplicate values. TGFα secretion as a function of postnatal age was analyzed using the *t* test. Significance was set at $p < 0.05$.

RESULTS

As predicted by previous studies on AM number after birth (13, 17, 19), there was an exponential increase in the number of AM during the first postnatal week with a linear increase

thereafter to adult levels (Fig. 1). Increased heterogeneity of AM density also was observed with advancing postnatal age (Fig. 2). AM isolated from animals less than 9–10 d of age were recovered solely in the lighter 1.06 g/dL Percoll gradient. At postnatal ages ≥9–10 d, AM were recovered from both 1.06 and 1.08 g/dL Percoll gradients. There was a transition from the predominate 1.06 subfraction to a combination of 1.06 and 1.08 subfractions. In 3/15 adult animals tested, AM also were isolated at the 1.10 g/dL Percoll interface layer, representing approximately 33% of the total AM isolated in those animals. This Percoll gradient fraction was not identified for any of the younger animals.

TGFα secretion by AM occurred at basal levels for all ages tested (Fig. 3). A statistically significant increase in TGFα secretion by ConA-treated AM from the 1.06 subfraction was first demonstrated at d 14 ($p < 0.02$). This increase above control TGFα secretion persisted with d 21 ($p < 0.009$) and d 28 samples ($p < 0.008$), but not with adult samples given the higher variability. An increase in TGFα secretion by ConA-treated AM above control also was displayed in the 1.08 subfractions of d 21 and 28 (juvenile) and adult rabbits, but not in the 1.10 subfraction that was present in 3/15 adult rabbits tested.

Analysis of the concentration of TGFα in media of control and ConA-stimulated AM by postnatal age was bimodal. The greatest concentrations (mean ng/mL ± SD) were in media of AM isolated from d 5–7 rabbits (3.1 ± 1.5 and 14.8 ± 6.8, respectively) and again at adult age (2.1 ± 0.8 and 21.3 ± 9.8, respectively).

Western blot analysis of control and ConA-treated AM samples are shown in Figures 4 and 5, respectively. In both control and ConA-treated AM samples, higher molecular mass proforms of TGFα (30–46 kD) were present at the earlier

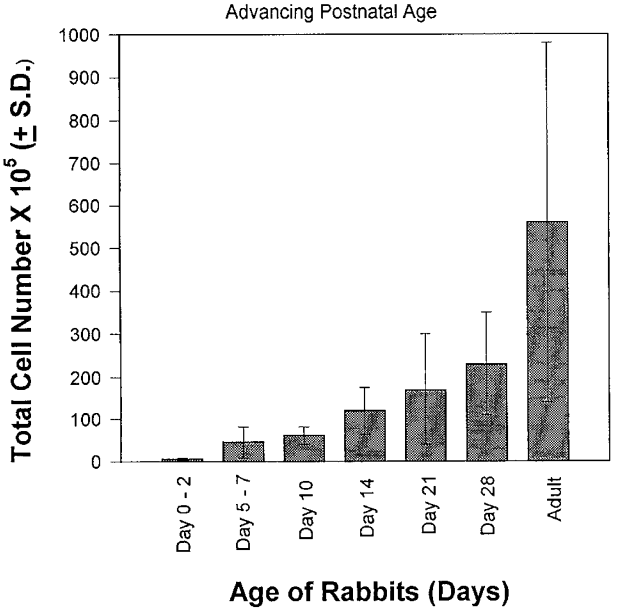

Figure 1. Bronchoalveolar lavage fluid cell counts. Total cell counts of bronchoalveolar lavage fluid (expressed as the mean ± SD) as a function of advancing postnatal age (days). The rabbits were sacrificed at d 0–2 (n = 11 litters), 5–7 (n = 15 litters), 9–10 (n = 6), 14 (n = 12), 21 (n = 16), 28 (n = 11), and at >4 mo (2–3-kg adult males; n = 15).

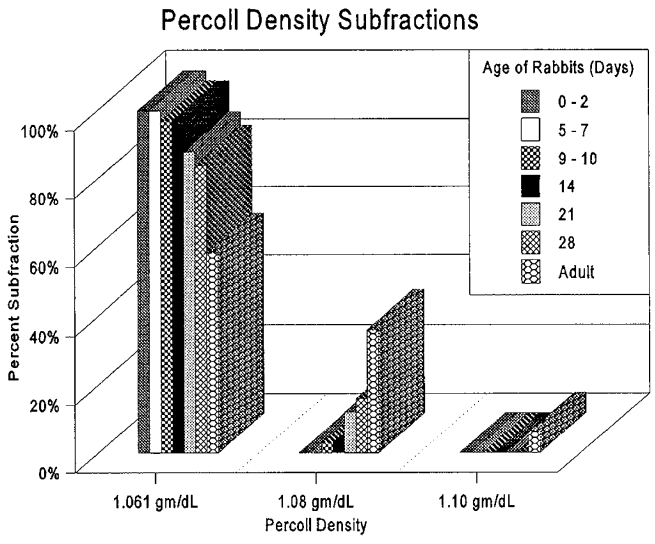

Figure 2. Postnatal rabbit AM Percoll density subfractions. AM were isolated by discontinuous Percoll densities of 1.061, 1.08, and 1.10 g/dL. The mean percent of AM isolated at each density subfraction is given for all ages tested.

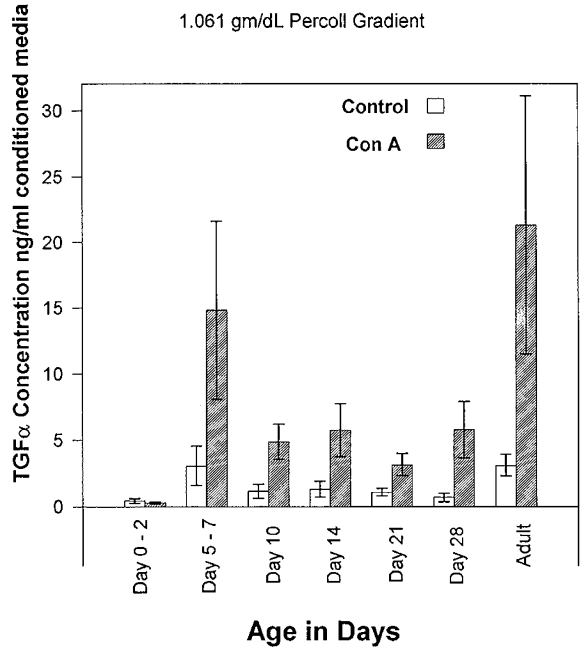

Figure 3. TGFα secretion by control and ConA-treated postnatal AM isolated in the 1.061 g/dL Percoll subfraction. AM TGFα concentration is expressed in ng/mL conditioned medium. Control and ConA-treated media were collected after 24 h cell culture.

postnatal ages (d 0–2 and 5–7), but were not detected in d 9/10 to adult samples. The mature (6 kD) form was detected only in juvenile (d 28) and adult ConA-treated AM media samples, and not in any of the control AM samples. The 14.3-kD moiety appeared to be the predominate isoform of ages d 9/10 to adult. Further, there appeared to be an increasing amount of this isoform detected with advancing postnatal age to adult at the 1.061 density and at the 1.08 density when present.

DISCUSSION

This study showed that AM isolated from newborn rabbits and those of advancing postnatal age secrete TGFα. There

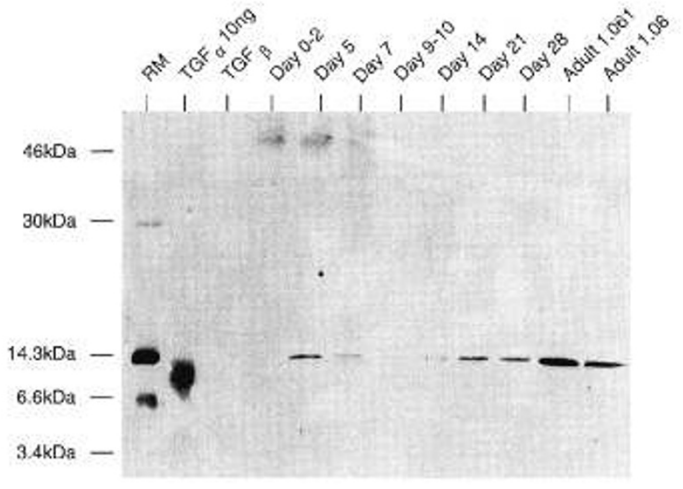

Figure 4. Western immunodetection of TGFα isoforms in AM control media samples. AM were isolated then placed in culture for 24 h in DME-F12 media alone. Subsequently, media samples were collected and concentrated 5-fold. Samples were electrophoresed through a 4% stacking and 15% resolving SDS-polyacrylamide gel. Appropriate controls were run. Proteins were transferred onto nitrocellulose using the semidry method and analyzed for the presence of TGFα isoforms through immunodetection. *Lane 1* from the left represents rainbow markers. *Lane 2* is TGFα, 10 ng (human recombinant 6 kD). *Lane 3* is TGFβ, 10 ng (human recombinant 25 kD). The subsequent lanes to the right are AM media samples isolated from rabbits of advancing postnatal age as indicated (d 0–2, 5, 7, 9–10, 14, 21, and 28, and adult). Not shown, DME-F12 medium alone was negative for TGFα bands.

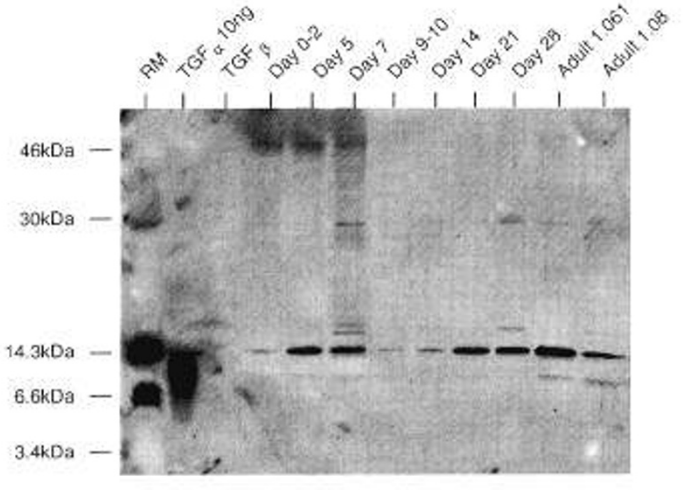

Figure 5. Western immunodetection of TGFα isoforms in AM-conditioned medium samples. AM were isolated then placed in culture for 24 h in DME-F12 medium with ConA (10 μg/mL). Subsequently, media samples were collected and processed for Western immunodetection of TGFα isoforms as described for control conditions (Fig. 4). *Lane 1* from the left represents rainbow markers. *Lane 2* is TGFα, 10 ng (human recombinant 6 kD). *Lane 3* is TGFβ, 10 ng (human recombinant 25 kD). The subsequent lanes to the right are AM media samples isolated from rabbits of advancing postnatal age as indicated (d 0–2, 5, 7, 9–10, 14, 21, and 28, and adult).

were qualitative and quantitative differences in the postnatal levels and biochemical forms of AM-derived TGFα. Whereas the 6-kD mature TGFα isoform was shown only in the media of ConA-stimulated juvenile and adult AM, the 14.3-kD moiety predominated with advancing postnatal age in both control and ConA samples. These age-dependent changes in TGFα secretion paralleled concurrent changes in the density at which the AM were isolated; with advancing postnatal age, there was a shift from the lighter 1.061 g/dL Percoll density to the 1.08 density.

Although it is unknown if the AM TGFα isoforms secreted during normal growth would change during times of stress, such as infection or hyperoxia, differential *in vitro* control and ConA-activated AM TGFα isoform secretion was demonstrated at all ages tested. Further, the 1.10 g/dL AM subfraction, isolated from 3/15 adult rabbits but not from earlier postnatal aged rabbits, failed to secrete TGFα *in vitro*. These differences between AM subfractions may represent an age- and activation-dependent mechanism by which selective secretion of TGFα is manifested in response to alterations in local tissue signals. Such differences in TGFα isoform secretion by AM may be important during injury in assisting the reparative process(es) of the lung.

The change from a homogeneous population of AM at the earlier postnatal ages to a mix of heavier and lighter density AM with advancing postnatal age may reflect AM maturation within the lung in response to local environmental cues rather than differential cell origin. Blusse van Oud Ablas *et al.* (33) showed that, during both the normal steady state and acute inflammation in the adult murine model, the vast majority of AM are derived from peripheral blood monocytes rather than local AM proliferation. Although not directly measured in this set of experiments, local *in vitro* AM proliferation has been shown by other investigators to be <2–5% (4, 33–35). Inasmuch as AM proliferation could potentially increase cell number and TGFα secretion, the contribution to the total TGFα concentration in the conditioned medium would be minimal. The major source of AM-derived TGFα is the mononuclear phagocyte, derived from peripheral blood monocytes similar in density and function until they are exposed to certain tissue-specific signals that allow differentiation (34). The influx of mononuclear phagocytes into the lung that occurs just before birth continues during the first postnatal weeks (14). During this rapid influx, the lung undergoes considerable change at the cellular and subcellular levels that likely influence monocyte differentiation into AM, and result in their subsequent activation and secretion of TGFα.

The interactional effect of lung epithelial cells (type II pneumocytes) and AM has been well documented in adult animal models (2–8). Only recently, studies have shown that TGFα has a stimulatory effect on newborn type II pneumocytes through activation of the EGF receptor (36). It is not known if there is differential activation of the EGF receptor by various higher molecular mass TGFα isoforms secreted by neighboring AM. Such differential activation, however, could result in greater control of cell proliferation. Alternatively, the transmembrane form of AM-derived TGFα may facilitate direct AM-type II cell interactions, thereby amplifying the initial signal at the EGF receptor through activation of a "second messenger" cascade.

The likely biologic effects of macrophage-derived TGFα on lung parenchyma come by extension from the study of macrophages isolated from other organ systems. Macrophage-

derived TGFα has been shown to mediate all three stages of dermal wound healing: reepithelialization, formation of granulation tissue, and induction of neovascularization (6). In addition, macrophage-derived TGFα directs an integral aspect of cellular metabolism by inducing the production of interferon-γ by lymphocytes and fibroblasts, collagenases, and stromelysin, and the synthesis of collagens in fibroblasts (6). Thus, macrophage-derived TGFα modulates both the normal processes of growth and the reepithelialization of tissues during injury. It is plausible that the TGFα isoforms secreted by AM serve a similar function in neonatal lung maturation and recovery following injury such as that induced by hyperoxia. Support for this hypothesis comes from Korfhagen *et al.* (37) who showed that respiratory epithelial cell expression of TGFα induced lung fibrosis in transgenic mice. The overexpression of TGFα by pulmonary epithelial cells in these mice led to a disruption of alveolar morphogenesis, producing fibrotic lesions that were presumably mediated by paracrine signaling between respiratory epithelial and interstitial cells of the lung. Although the effect of AM-derived TGFα in this model has not been addressed, it is likely that these cells could contribute to the derangement noted in this system.

The TGFα isoforms previously reported have ranged from 5 to 22 kD. The largest molecular mass TGFα isoform expected from primary translational production based on sequence is 26 kD (38, 39). The variation in TGFα molecular mass moieties presumably reflects differential glycosylation and proteolytic cleavage, as well as dimerization of the binding proteins (38–40). Humphreys-Beher *et al.* (41) reported TGFα isoforms present in mouse saliva that included a 30–46-kD moiety that they attributed to nonspecific binding. We report similar higher molecular mass isoforms. The absence of these higher molecular mass isoforms from later postnatal age AM media samples, the minimal staining of rainbow molecular mass markers, and negative secondary control support the premise that these isoforms are true TGFα-like moieties reacting with the TGFα MAb. Similar higher molecular mass TGFα isoforms have been identified in the medium of human milk macrophages in our laboratory with only the 6-kD isoform identified in human milk supernatant (42). It appears, therefore, that higher molecular mass isoforms >22 kD are secreted by AM and may represent aggregation at disulfide bonds, or as yet, another unidentified member of the EGF family with structural homology to TGFα.

In summary, the qualitative and quantitative differences in the postnatal levels and biochemical forms of AM-derived TGFα may result in varying stimulatory effects on lung epithelium and may contribute to the disparity of injury responses noted between newborns and adults. Further investigation is necessary to determine the *in vivo* effect of the various AM-derived TGFα isoforms on lung epithelial maturation and recovery after injury. Experiments to isolate and delineate the biochemical and cellular effects of the various higher molecular mass TGFα isoforms are underway.

REFERENCES

1. Madtes DK, Raines EW, Sakariassen KS, Assoian RK, Sporn MB, Bell GI, Ross R 1988 Induction of transforming growth factor-α in activated human alveolar macrophages. Cell 53:285–293
2. Brandes ME, Finkelstein JN 1990 The production of alveolar macrophage-derived growth-regulating proteins in response to lung injury. Toxicol Lett 54:3–22
3. Brandes ME, Finkelstein JN 1990 Alveolar macrophage production of type II cell growth modulators following *in vivo* hyperoxia. Toxicologist 10:101(abstr. 403)
4. Brandes ME, Finkelstein JN 1989 Stimulated rabbit alveolar macrophages secrete a growth factor for type II pneumocytes. Am J Respir Cell Mol Biol 1:101–109
5. Driscoll KE, Maurer JK 1991 Cytokine and growth factor release by alveolar macrophages: Potential biomarkers of pulmonary toxicity. Toxicol Pathol 19:398–405
6. Rappolee DA, Werb Z 1992 Macrophage-derived growth factors. Curr Top Microbiol Immunol 181:87–139
7. Nathan CF 1987 Secretory products of macrophages. J Clin Invest 79:319–326
8. Johnston RB 1988 Monocytes and macrophages. Current concepts: Immunology. N Engl J Med 318:747–751
9. Kelley J 1990 State of the art: Cytokines of the lung. Am Rev Respir Dis 141:765–788
10. Luetteke NC, Lee DC 1990 Transforming growth factor alpha: expression, regulation and biological action of its integral membrane precursor. Cancer Biol 1:265–275
11. Kudlow JE, Bjorge JD 1990 TGFα in normal physiology. Cancer Biol 1:293–302
12. Derynck R 1992 The physiology of transforming growth factor-α. Adv Cancer Res 58:27–51
13. Lyons RM, Moses HL 1990 Transforming growth factors and the regulation of cell proliferation. Eur J Biochem 187:467–473
14. Zeligs B, Nerurkar LS, Bellanti JA 1977 Maturation of the rabbit alveolar macrophage during animal development. I. Perinatal influx into alveoli and ultrastructural differentiation. Pediatr Res 11:197–208
15. Bellanti JA, Nerurkar LS, Zeligs BJ 1979 Host defenses in the fetus and neonate: Studies of the alveolar macrophage during maturation. Pediatrics 64:726–739
16. Sherman M, Goldstein E, Lippert W, Wennberg R 1977 Neonatal lung defense mechanisms: A study of the alveolar macrophage system in neonatal rabbits. Am Rev Respir Dis 116:433–440
17. Kurland C, Cheung ATW, Miller ME, Ayin SA, Cho MM, Ford EW 1988 The Ontogeny of pulmonary defenses: Alveolar macrophage function in neonatal and juvenile rhesus monkeys. Pediatr Res 23:293–297
18. Sieger L 1978 Pulmonary alveolar macrophages in pre- and post-natal rabbits. J Reticuloendothel Soc 23:389–395
19. Kradin RL, McCarthy KM, Schneeberger EE 1966 Opsonic receptor function is reduced on the surface of newborn alveolar macrophages. Am Rev Respir Dis 133:238–244
20. Jacobs RF, Wilson CB, Palmer S, Springmeyer SC, Henderson WR, Glover DM, Kessler Jr DL, Murphy JH, Hughes JP, Van Belle G, Chi EY, Hodson WA 1985 Factors related to the appearance of alveolar macrophages in the developing lung. Am Rev Respir Dis 131:548–553
21. Hardy B, Skutelsky E, Globerson A, Danon D 1976 Ultrastructural differences between macrophages of newborn and adult mice. J Reticuloendothel Soc 19:291–299
22. Nerurkar LS, Zeligs BJ, Bellanti JA 1977 Maturation of the rabbit alveolar macrophage during animal development. II. Biochemical and enzymatic studies. Pediatr Res 11:1202–1207
23. Zeligs BJ, Nekurkar LS, Bellanti JA 1977 Maturation of the rabbit alveolar macrophage during animal development. III. Phagocytic and bactericidal functions. Pediatr Res 11:1208–1211
24. Sherman M, Goldstein E, Lippert W, Wennberg R 1977 Neonatal lung defense mechanisms: A study of alveolar macrophage system in neonatal rabbits. Am Rev Respir Dis 116:433–440
25. Papadimitriou JM, Ashman RB 1989 Macrophages: Current views on their differentiation, structure and function. Ultrastruct Pathol 13:343–372
26. Nakstad B, Scient C, Lyberg T 1989 Subpopulations of human lung alveolar macrophages: Ultrastructural features. Ultrastruct Pathol 13:1–13
27. Sibille Y, Reynolds HY 1990 Macrophages and polymorphonuclear neutrophils in lung defense and injury. Am Rev Respir Dis 141:471–501
28. Brannen AL, Chandler DB 1988 Alveolar macrophage subpopulations responsiveness to chemotactic stimuli. Am J Pathol 132:161–166
29. Zwilling BS, Campolito LB, NA Reiches 1982 Alveolar macrophage subpopulations identified by differential centrifugation on a discontinuous albumin density gradient. Am Rev Respir Dis 125:448–452
30. Miller RG, Phillips RA 1970 Separation of cells by velocity sedimentation. J Cell Physiol 73:191–202
31. Laemmli UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
32. Towbin H, Staehelin T, Gordon J 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354
33. Blusse van Oud Albas A, Van Der Linden-Schrever B, Van Furth R 1983 Origin and kinetics of pulmonary macrophages during an inflammatory reaction induced by intra-alveolar administration of aerosolized heat-killed BCG. Am Rev Respir Dis 128:276–281
34. Bitterman PB, Saltzman LE, Adelberg S, Ferrans VJ, Crystal RG 1984 Alveolar macrophage replication. One mechanism for the expansion of the mononuclear phagocyte population in the chronically inflamed lung. J Clin Invest 74:460–469
35. Elias JA, Zuhier RB, Schrieber AD, Leff JA, Daniele RP 1985 Monocyte inhibition of lung fibroblast growth: Relationship to fibroblast prostaglandin production and density-defined monocyte subpopulations. J Leukocyte Biol 37:15–28
36. Ryan RM, Mineo-Kuhn M, Kramer CM, Finkelstein JN 1994 Growth factors alter neonatal type II alveolar epithelial cell proliferation. Am J Physiol 226:L17–L22

37. Korfhagen TR, Swantz RJ, Wert SE, McCarty JM, Keriakian CB, Glasser SW, Whitsett JA 1994 Respiratory epithelial cell expression of human transforming growth factor-α induces lung fibrosis in transgenic mice. J Clin Invest 93:1691–1699
38. Qian JF, Lazar-Wesley E, Breugnot C, May E 1993 Human transforming growth factor α: Sequence analysis of the 4.5-kb and 1.6-kb mRNA species. Gene 132:291–296
39. Russell WE, Dempsey PJ, Sitaric S, Peck AJ 1993 Transforming growth factor-α (TGFα) concentrations increase in regenerating rat liver: Evidence for a delayed accumulation of mature TGFα. Endocrinology 133:1731–1738
40. Coffey RJ, Romano M, Polk WH, Dempsey PJ 1992 Roles for transforming growth factor-α in gastric physiology and pathophysiology. Yale J Biol Med 65:693–704
41. Humphreys-Beher MG, Macauley SP, Chegini N, vanSetten G, Purushotham K, Stewart C, Wheeler TT, Schultz GS 1994 Characterization of the synthesis and secretion of transforming growth factor-α from salivary glands and saliva. Endocrinology 134:963–970
42. Wagner CL, Forsythe DW 1994 Variation in the biochemical forms of transforming growth factor-α (TGFα) present in human milk and secreted by human milk macrophages (HMM). Pediatr Res 35:137A(abstr)