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Development of Chemotactic Factor Inactivation– a Fetal Lamb Model

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Summary

To study the developmental pattern of chemotactic factor inactivator (CFI) activity, although avoiding the effects of labor and delivery, serial blood samples were obtained from eight chronically catheterized lamb fetuses, 13 pregnant, and 13 nonpregnant ewes. The biologic profiles of adult sheep neutrophils in response to sheep C_5-derived chemotactic fractions ($C_{5\text{-fr}}$) were determined by the chemotactic and lysosomal enzyme release assays. CFI activity in sheep plasma was characterized and measured by the % inhibition of $C_{5\text{-fr}}$-induced neutrophil lysosomal enzyme release.

The chemotactic and enzyme release profiles of sheep neutrophils paralleled those of human neutrophils. Sheep plasma was shown to contain a CFI-like activity similar to that in human plasma.

Mean CFI activity was higher in the plasma of pregnant ewes as compare to nonpregnant ewes (7.60 *versus* 5.15%, $P < 0.001$). Higher CFI levels (39.5%) were shown in the plasma of chronically catheterized fetuses early in the third trimester of pregnancy (109 days gestational age). These fetal levels decreased progressively to attain normal adult levels at fetal maturity (147 days gestational age) and no significant changes were noted at or after birth. Because identical changes occurred in all chronically catheterized fetuses, a developmental pattern was defined for CFI. Control CFI levels from six acutely catheterized fetuses did not differ from those predicted by the developmental pattern. Thus, the changes in CFI activity were not related to the chronic catheterization or to events surrounding labor and delivery; rather CFI levels correlated best with fetal immaturity.

CFI levels correlated inversely with ability of zymosan activated plasma to induce neutrophil chemotaxis ($r = -0.96$, $P < 0.01$), suggesting that CFI plays a major role in the regulation of leukocytes chemotaxis.

These data confirm previous findings in human neonates and provide a model for future studies. Further studies into the biology of the changes in CFI activity in the fetus and the influence of such high risk factors as prematurity, fetal distress or premature rupture of membranes should enhance our understanding of the immunocompromised neonate.

Complement activation in serum and plasma results in the generation of C_5-derived chemotaxins ($C_{5\text{-fr}}$) (2, 20), which induce neutrophil (2), monocyte (3) and lymphocyte chemotaxis (4). Leukocyte chemotactic responses are dose-dependent (12), thus related to chemotatic factor generation, and can be adversely affected by the chemotactic factor inactivator (CFI) (13, 15, 17) and other chemotactic inhibitors (16). CFI activity has been demonstrated in normal adult human plasma (5, 13) and serumd (5, 17). Higher levels were shown in patients with malignancies (17), liver disease (10) and in newborn infants (14). The ability of activated human plasmas to induce neutrophil chemotaxis correlated inversely with their CFI activity in both the adults and newborns ($r = -0.956$, $P < 0.001$) (14); similarly, the ability to mobilize inflammatory cells in both the acute (neutrophil-mediated) and delayed (mononuclear-mediated) inflammatory reactions was decreased in patients with high CFI levels (18). Thus, high CFI levels may contribute to an increased susceptibility to infections.

The mechanisms whereby CFI inactivates chemotaxins remain obscure; CFI has clearly been shown to inactivate chemotactic factors such as $C_{5\text{-fr}}$, bacterial- and lymphocyte-derived factors and some synthetic peptides (15, 19). Although CFI activity did not directly alter leukocyte responsiveness to the chemotactic factors, we have recently demonstrated that product(s) of $C_{5\text{-fr}}$ inactivation by CFI inhibit neutrophil chemotactic and enzyme release responses to unaltered $C_{5\text{-fr}}$ (13). Better understanding of the mechanisms and clinical relevance of CFI activity have been hampered by the difficulties in the purification of CFI (9) and the lack of animal model.

Our objectives in this paper were to describe an animal model for both the healthy adult and the neonate using adult ewes and chronically catheterized lamb fetuses. A CFI activity similar to that seen in the humans was demonstrated in fetal and adult sheep plasma. The effects of pregnancy on CFI levels were analyzed and CFI activity in the chronically catheterized fetus was serially tested and correlated with the gestational age.

MATERIALS AND METHODS

Animal model. Nonpregnant and pregnant mixed breed Dorset-Suffolk ewes were obtained from local sources and housed at the University of Iowa Animal Quarters throughout the study period. The gestational ages of the fetuses were known based upon the induced ovulation techniques (1, 7). A heparinized catheter was placed in the femoral artery of the fetuses at various gestational ages under general anesthesia of the ewe (8). A group of eight fetuses were catheterized at the ages of 101–107 days and serially evaluated. Another group of six fetuses was catheterized at the ages of 124–137 days; however, only the blood samples obtained at surgery were tested in these actutely catheterized fetuses, to avoid the possible effects of chronic catheterization.

Blood collection. Venous blood was obtained from the ewes and newborn lambs by venipuncture and mixed with sodium citrate (0.38% w/v final concentration). From catheterized fetuses the sample was collected via the catheter; 1 ml of blood was withdrawn to clear the catheter of saline, 9 ml citrated blood was then obtained as previously described (8). Following delivery, blood samples were obtained from these lambs by venipuncture. Plasma was removed by centrifugation at 1800 × *g* for 30 min and stored at −70°C in aliquots. Serum samples were similarly obtained after allowing venous blood to clot at 22°C for 1 hr.

Preparations of neutrophil suspensions. Neutropohils were separated from citrated blood of adult nonpregnant ewes. Methods for cell separation were similar to those used for human neutrophils (12, 13) with only minor changes: citrated venous blood was mixed (3:1 v/v) with normal saline containing 2% Bovine serum albumin (BSA) and centrifuged at 300 X *g* for 15 min. The buffy coat was suspended in 10 ml saline, layered over a Ficoll-Hypaque gradient and centrifuged at 300 X *g* for 45 min. Red blood cells were

removed by hypotonic lysis and the neutrophils were washed once with 140 mM NaCl. All the above steps were performed at 4°C and resulted in 40% cell recovery, with >95% viable neutrophils by exclusion of trypan blue dye.

Neutrophils were suspended in Gey's solution, containing 2% BSA at 50×10^6 cells/ml when prepared for the chemotactic assays, and in Hank's balanced salt solution (HBSS) containing 4 μg/ml cytochalasin B at 10×10^6/ml when prepared for enzyme release inhibition assays (13).

Preparation of chemotaxins. Zymosan activated plasma (ZAP) and serum (ZAS) were prepared using standard techniques (6). One mg zymosan in 0.1 ml H_2O was mixed with 1 ml sheep plasma or serum and incubated at 39°C for 30 min. Zymosan was then removed by centrifugation and the activated material was heated at 56°C for 30 min.

C_5-derived chemotactic fractions ($C_{5\text{-fr}}$) were also prepared using standard methods (5). Pooled sheep serum was activated with zymosan in the presence of 1.0 M ϵ-amino-*n*-caproic acid and the chemotactic fractions were separated by sephadex G-100 chromatography, concentrated to ½ the original volume and dialyzed against water. A batch of $C_{5\text{-fr}}$ was prepared, standardized by defining the quantities capable of inducing maximal chemotactic and enzyme release responses, then stored in aliquots at −70°C.

Chemotaxis under agarose. Previously described agarose tests (12) were used to quantify sheep neutrophil responses to sheep ZAP and $C_{5\text{-fr}}$. In these assays, the gel contained 0.5% agarose and 2% BSA in Gey's solution. Three wells, 3 mm in diameter, 3 mm apart were made in a row. Ten μl cell suspensions containing 0.5×10^6 neutrophils were placed in the lateral wells. The medial well received 20 μl of a mixture containing Gey's solution and 0, 6, 8, 10, 12, 14, 16, or 20 μl $C_{5\text{-fr}}$ to determine the chemotactic dose-response curve. In standard chemotactic assays, the medial well received 20 μl Gey's solution containing 0 or 10 μl ZAP. Migration distances of neutrophils towards the central well in the presence or absence of $C_{5\text{-fr}}$ were determined after a 3 h incubation at 39°C to measure chemotaxis and spontaneous migration. The chemotactic differential (chemotaxis minus spontaneous migration) was used to define the chemotactic activity of a test sample. Results were expressed as % of the maximal response in the dose response assays and as % of the response to 10 μl of pooled ZAP in the standard assays. All assays were performed in quadruplicate.

Enzyme release assays. Release of the lysosomal enzyme, N-acetyl-β-D-glucosaminidase, from cytochalasin B-treated neutrophils was measured as previously described with minor modifications (5, 13): 100 μl cell suspensions containing 10^6 cytochalasin B-treated neutrophils were mixed with 150 μl HBSS containing $C_{5\text{-fr}}$ quantities equivalent to 0, 20, 25, 30, 35, 40, 45, and 50 μl and incubated for 5 min at 39°C. The neutrophils were removed by centrifugation at $200 \times g$ and the enzymatic activity was measured by incubating 100 μl of the supernatant with 8000 μl of 4 nM solution of *p*-nitrophenyl-N-acetyl-β-D-glucosaminide, in 0.05 M sodium citrate buffer (pH 4.5), at 39°C for 1 h. The reaction was stopped by adding 1 ml of glycine buffer (pH 10.5) and the absorbance was determined at 410 nm. In these assays, 35 μl of $C_{5\text{-fr}}$ constantly induced maximal enzyme release, consequently this quantity was used in enzyme release inhibition assays.

Lysosomal enzyme release inhbition assays. CFI activity inhibits the $C_{5\text{-fr}}$-induced neutrophil lysosomal enzyme release (5). This inhibition is dose-dependent, time-dependent and irreversible (5, 13). Furthermore, CFI activity is heat labile. The enzyme release inhibition assay (5) was modified (13) to define CFI activity and its characteristics in adult and fetal plasma.

Dose-dependent curves were determined by premixing 35 μl $C_{5\text{-fr}}$ with 115 HBSS containing plasma quantities equivalent to 0, 0.001, 0.01, 1.0, 2.0, and 10 μl. The mixtures were incubated at 39°C for 30 min then assayed for lysosomal enzyme release. Inhibition of lysosomal enzyme release was determined as follows:

$$\%\ \text{Inhibition (CFI activity)} = \frac{\text{LER}_{max} - \text{LER}_{sample}}{\text{LER}_{max}} \times 100$$

Where LER_{max} was the enzyme release induced by 35 μl $C_{5\text{-fr}}$ and LER_{sample} the enzyme release induced by 35 μl $C_{5\text{-fr}}$ that had been preincubated with plasma.

The time dependency of the inhbition of $C_{5\text{-fr}}$ by plasma was determined by preincubating 35 μl $C_{5\text{-fr}}$ with 115 μl HBSS containing 10 μl plasma for 0, 5, 10, 15, 20, 25 and 30 min at 39°C before testing for inhibition of lysosomal enzyme release. To evaluate the irreversible effects of CFI on $C_{5\text{-fr}}$, the plasma-$C_{5\text{-fr}}$ mixture was incubated at 39°C for 30 min, then heated at 56°C for 120 min before testing for enzyme release inhibition. Finally, the heat lability of CFI in plasma was determined by preheating the plasma for 0–120 min before mixing with $C_{5\text{-fr}}$. The mixtures were then preincubated at 39°C for 30 min and assayed for enzyme release inhibition.

Standard enzyme release inhibition assays were performed as previously described (13): 35 μl $C_{5\text{-fr}}$ were preincubated with 0.01 μl plasma in 115 μl HBSS for 30 min at 39°C, then assayed for enzyme release inhibition. Thus, CFI activity in a test plasma was defined by the % inhibition of maximal enzyme release induced by 0.01 μl plasma.

All enzyme release and enzyme release inhibition assays were run in triplicate.

Statistical methods. The two-sample *t* test was used to compare mean CFI levels in the plasma of pregnant and nonpregnant ewes. Polynomial regression was used to describe the relationship between CFI level and gestational age in the chronically catheterized fetuses. The residual S.D. was used to describe the variability about the regression line; this statistic estimates the variability of CFI level in these fetuses about the predicted mean CFI level for a given gestational age. The squared multiple correlation coefficient (r^2) is presented as a measure of the goodness-of-fit of the derived regression line; this statistic is interpreted as the proportion of variability which is explained by the regression. A value near 100% indicates that the majority of the overall variability observed in CFI is accounted for (explained) by the gestational age.

In order to determine whether chronic catheterization affected CFI levels, control data was obtained on six fetuses that were catheterized shortly before delivery. The observed values in these control animals were compared to values predicted from the regression equations developed on the chronically catheterized animals, using a one-sample *t* test on the differences between observed and predicted values. A nonsignificant *t* test indicates that, on the average, the deviations from predicted values were not different from zero, suggesting that chronic catheterization did not affect CFI levels in the fetus.

Pre- and postdelivery CFI levels were compared using the paired *t* test. The strength of the association between CFI level and the chemotactic activity of zymosan activated plasma was measured using the Pearson correlation coefficient.

RESULTS

Neutrophil dose-response curves in chemotaxis and lysosomal enzyme release assays. Neutrophils from three healthy nonpregnant ewes were assayed, on different days, in chemotaxis and enzyme release assays (Fig. 1). Dose-response profiles were similar in both assays. Neutrophil responses increased sharply to a maximal level, then decreased as $C_{5\text{-fr}}$ concentrations were increased. Maximal responses always occurred at 14 μl and 35 μl $C_{5\text{-fr}}$ respectively in the chemotaxis and enzyme release assays.

Maximal chemotactic responses (chemotactic differentials) varied between 2.2 and 2.4 mm and maximal lysosomal enzyme release responses varied between 0.220 and 0.260 absorbance units. S.D. of quadruplicate chemotactic measurements were within 6% of the mean, and S.D. of triplicate enzyme release measurements were within 5% of the mean.

CFI activity in sheep plasma. As shown in Figure 2, preincubation of $C_{5\text{-fr}}$ with plasma induced a dose-dependent inhibition of the chemotactic and lysosomal enzyme releasing activities. As previously shown in human plasma (13), the inhibition dose-response curves were sigmoid with the linear segments corresponding to the logarithm of plasma quantities ranging between 0.01–1.0 μl. This inhibitory activity became apparent only after a

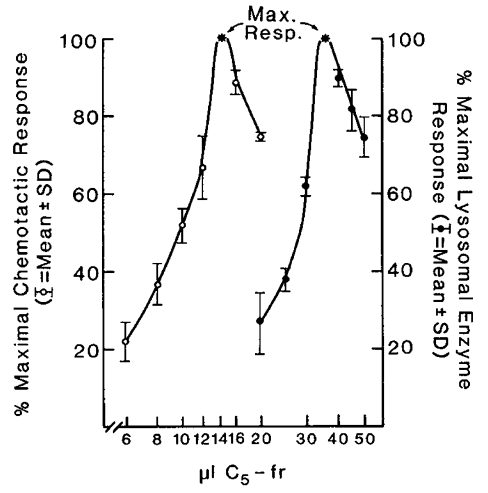

Fig. 1. Sheep neutrophil chemotaxis and lysosomal enzyme release in response to sheep $C_{5\text{-}fr}$. Neutrophils of three adult ewes were tested on 3 different days.

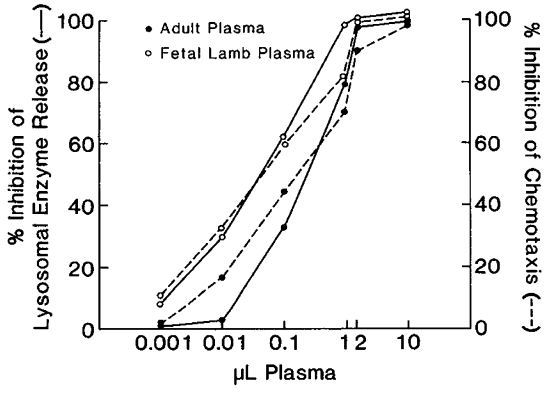

Fig. 2. Inhibition of $C_{5\text{-}fr}$-induced lysosomal enzyme release and chemotaxis. A measure of CFI activity. Similar results were obtained using three adult and three fetal lamb plasmas.

preincubation period of 10 min and attained a maximum by 25–30 min (Fig. 3) in both adult and fetal plasmas. Since preincubation of the plasma-$C_{5\text{-}fr}$ mixture was performed before adding the neutrophil suspension, these results suggested that neutrophil responsiveness was not directly affected by the presence of plasma in the reaction mixture. Rather, the inhibitory activity was directed at $C_{5\text{-}fr}$. This was demonstrated further in Table 1: when 2 μl adult or fetal plasma were preincubated with $C_{5\text{-}fr}$, the enzyme releasing activity of $C_{5\text{-}fr}$ was totally inhibited (99–100%). However, minimal inhibition of the enzyme release was demonstrated when 10 μl adult or fetal plasma were preincubated with the neutrophils (7% with adult plasma and 5% with fetal plasma. Not statistically significant by the unpaired *t* test).

Heating the plasma at 56°C for 0–120 min before its preincubation with $C_{5\text{-}fr}$ resulted in a progressive loss of inhibitory activity in adult and fetal plasma. Virtually all inhibitory activity (97–98%) was lost after 110–120 min (Fig. 4 and Table 2). However, when the plasma was first incubated with $C_{5\text{-}fr}$ for 30 min at 39°C, then heated at 56°C for 120 min to destroy the inhibitory activity in the plasma, the inhibition of enzyme release was not reversed (Table 2).

Thus, these studies demonstrated that, at the plasma concentrations used, the enzyme release inhibiting activity of adult sheep and fetal lamb plasma was heat labile, $C_{5\text{-}fr}$-directed, time-dependent and its effects were irreversible. These features are characteristic of CFI activity (5, 6, 14). A difference in heat lability of CFI between adult and fetal plasma, similar to the difference in heat lability between human adult and newborn plasma (13, 14), is seen. Reasons for this difference remain obscure and may be related to a difference in concentration (13, 14).

CFI levels in adult ewes, effects of pregnancy. CFI activity was measured in 13 nonpregnant ewes using plasma quantities equivalent to 0.01 μl. These plasmas inhibited enzyme release by 5.15% ± 2.92 (mean ± S.D.). When aliquots of the same plasma were

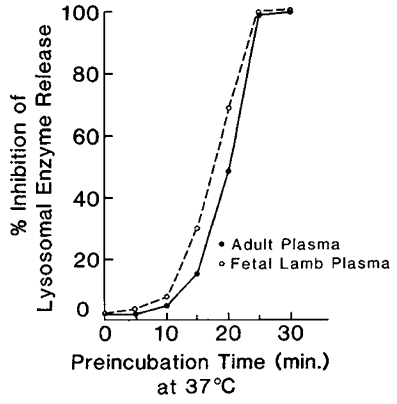

Fig. 3. Inhibition, by plasma, of $C_{5\text{-}fr}$-induced lysosomal enzyme release as a function of preincubation time at 39°C. Preincubation of $C_{5\text{-}fr}$ with plasma was performed before neutrophil suspensions were added. *Each data point* represents a mean of three experiments.

Table 1. *Characteristics of CFI activity in sheep plasma: I-inhibition is time dependent and directed at $C_{5\text{-}fr}$*

Initial mixture	Incubation time (min) at 39°C	Material added after incubation	% (mean ± S.D.) inhibition of lysosomal enzyme release (n = 5)
35 μl $C_{5\text{-}fr}$ + 2 μl adult plasma	0	10^6 neutrophils (in 100 μl Hanks')	1 ± 1
35 μl $C_{5\text{-}fr}$ + 2 μl adult plasma	30	10^6 neutrophils (in 100 μl Hanks')	99 ± 3
35 μl $C_{5\text{-}fr}$ + 2 μl fetal plasma	0	10^6 neutrophils (in 100 μl Hanks')	2 ± 1.5
35 μl $C_{5\text{-}fr}$ + 2 μl fetal plasma	30	10^6 neutrophils (in 100 μl Hanks')	100 ± 2
10^6 neutrophils + 10 μl adult plasma	0	35 μl $C_{5\text{-}fr}$	7 ± 2
10^6 neutrophils + 10 μl adult plasma	30	35 μl $C_{5\text{-}fr}$	4.5 ± 4
10^6 neutrophils + 10 μl fetal plasma	0	35 μl $C_{5\text{-}fr}$	6 ± 4
10^6 neutrophils + 10 μl fetal plasma	30	35 μl $C_{5\text{-}fr}$	5 ± 4

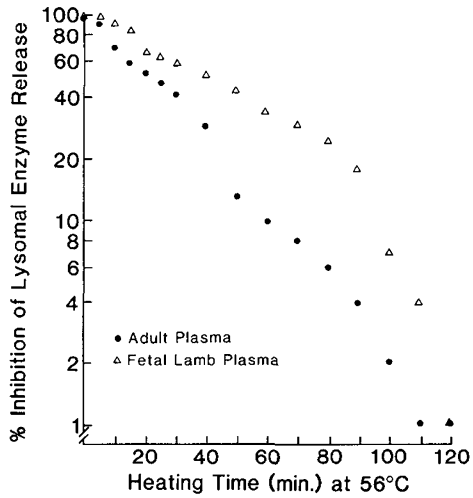

Fig. 4. Heat lability of the inhibitory activity in plasma. *Each data point* represents a mean of three experiments.

Table 2. *Characteristics of CFI activity in sheep plasma: II-inhibitory activity is heat labile and its effects are irreversible*

Reaction mixtures (in 150 μl Hanks')	Incubation and heat treatment	% inhibition of lysosomal enzyme release
35 μl $C_{5\text{-fr}}$	(1) 30 min at 39°C	0 (control)
35 μl $C_{5\text{-fr}}$	(1) 30 min at 39°C; 120 min at 56°C	2
35 μl $C_{5\text{-fr}}$ + 2 μl adult plasma	(1) 30 min at 39°C	99
35 μl $C_{5\text{-fr}}$ + 2 μl adult plasma	(1) 30 min at 39°C; (2) 120 min at 56°C	98
35 μl $C_{5\text{-fr}}$ + 2 μl adult plasma (heated)[1]	(1) 30 min at 39°C	1
35 μl $C_{5\text{-fr}}$ + 2 μl fetal plasma	(1) 30 min at 39°C	100
35 μl $C_{5\text{-fr}}$ + 2 μl fetal plasma	(1) 30 min at 39°C; (2) 120 min at 56°C	100
35 μl $C_{5\text{-fr}}$ + 2 μl fetal plasma (heated)[1]	(1) 30 min at 39°C	2

[1] 120 min at 56°C.

tested on 5 different days, CFI activity was 3, 4, 7, 5, and 3%. S.D. of triplicate measurements on the same day were constantly below 7% of the mean.

CFI activity in plasma from 13 pregnant ewes during the third trimester of pregnancy was higher than that in plasma of nonpregnant ewes (mean difference = 7.16% 2.11, t = 2.795, $P < 0.001$). However, while the means were significantly different, the data showed a great overlapping of CFI levels.

CFI levels in the fetus. CFI activity was determined in the plasma of eight chronically catheterized fetuses collected during the last 40 days of gestation; precisely between the age of 109 days and birth. As shown in Figure 5, CFI levels ranged between 34–44% in the 109-day-old fetuses and decreased progressively thereafter to attain adult CFI levels (5%) in the term fetus (gestational age 147 days). Inasmuch as changes in CFI activity were similar in all fetuses, a developmental pattern was developed. Polynomial regression was used to develop a curve to predict CFI activity as a function of gestational age. Analyses indicated that a quadratic polynomial was the best fitting curve. The equation for this curve was:

$$\text{CFI (\%)} = 312.2 - 3.6649\ (\text{GA}) + (0.010688)\ (\text{GA})^2$$

where GA was the gestational age. This curve fitted the data well; the residual S.D. (a measure of the variability about regression)

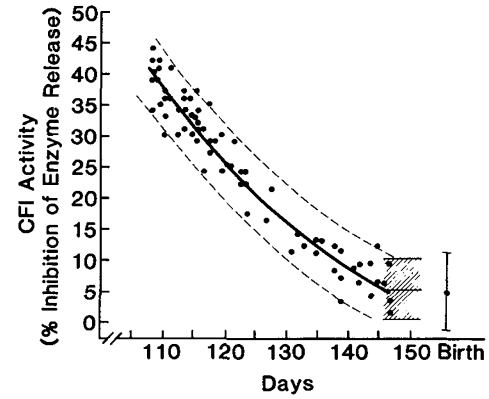

Fig. 5. CFI activity in the fetus during the third trimester of pregnancy (mean ± 2 S.D.). *Bar*, mean ± 2 S.D. at birth. *Shaded areas*, mean ± 2 S.D. in nonpregnant ewes.

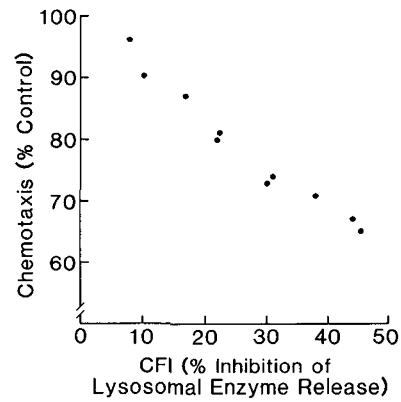

Fig. 6. Correlation between CFI activity and chemotactic activity in fetal lamb plasma.

was 2.9 units. The analyses yielded $r^2 = 0.933$ ($P < 0.001$) indicating that 93.3% of the variability in CFI activity was explained by the regression.

Effects of chronic catheterization. To determine whether changes in CFI activity during the third trimester of gestation were related to the chronic catheterization, another group of six fetuses was allowed to attain the gestational ages of 124, 125, 129, 132, and 137 days before catheterization. A blood sample was obtained during surgery from the femoral artrery of each fetus immediately after the catheter was placed. The CFI activity observed in each plasma sample was compared to the CFI activity predicted by the developmental pattern in the chronically catheterized fetuses, using a one-sample *t* test. The mean difference between predicted and observed values was 2.4 ± 3.1 (mean ± S.D.) yielding t = 1.879 ($P = 0.12$). Thus, the changes in CFI activity were not related to chronic catheterization.

Effects of labor and delivery. Delivery in the sheep occurs at about 145 days of gestation. Delivery of chronically catheterized fetuses occurred between 139–151 days. Blood samples were obtained by venipuncture within 24 h of birth. Plasma CFI activity was 6% ± 4.5 (mean ± S.D.) in the neonates and did not differ significantly from CFI activity in fetal plasma that was obtained shortly before birth when compared by the paired *t* test. Thus, no significant changes in CFI activity were demonstrated in relation to either labor or delivery.

Correlation between CFI activity and the chemotactic activity of activated plasma. Ten plasma samples were tested for CFI activity, then activated with zymosan and tested for their ability to induce neutrophil chemotaxis. As shown in Figure 6, a reciprocal relationship was shown between CFI and chemotactic activities (r = −0.96); this relationship was similar to that in human plasma (13, 14).

DISCUSSION

The development of chemotactic, enzyme releasing and enzyme-release inhibition assays has made possible the characterization of neutrophil dose-response profiles to $C_{5\text{-}fr}$ in the sheep, and the demonstration, characterization and quantitation of CFI activity in adult sheep, pregnant ewes and lamb fetuses. Furthermore, the use of the chronic fetal lamb model permitted studies of CFI changes during the third trimester of pregnancy. Profiles of the dose-response curves of adult nonpregnant sheep neutrophils to $C_{5\text{-}fr}$ (Fig. 1) paralleled those of human neutrophils (13). CFI activity, defined by its heat lability (Fig. 2), time dependency (Fig. 3) and the irreversibility of its effects (Table 2) accounted for >98% of the $C_{5\text{-}fr}$-directed inhibitory activity of adult sheep and fetal lamb plasma; similar results were previously obtained with human plasma (13, 14). The lack of difference in CFI activity between the chronically catheterized and the acutely catheterized fetuses suggested that chronic catherization did not influence the developmental pattern of CFI activity. The pattern of development for CFI was similar in all eight fetuses as shown by the ability of a single regression curve to explain 93.3% of the changes in activity (Fig. 5). Consequently, the sheep model can provide insight into the changes in CFI during fetal maturation.

CFI activity in human cord blood plasma was previously shown to be higher than that in human adult plasma and seemed to account for most of the difference in chemotactic activities between cord and adult plasmas (14). The relationship of neonatal CFI levels to events surrounding labor and delivery and to maternal CFI levels remained obscure. The mechanisms whereby CFI activity is increased in the human neonate and the effects of labor and delivery, prematurity, and other neonatal high risk factors on CFI levels remain unknown.

Although CFI levels in pregnant ewes were higher than those in nonpregnant ewes, the amount of overlap was great. Conversely, CFI levels in the 109-day-old fetuses were significantly higher than and did not overlap with those in the mothers. These levels decreased steadily to attain normal adult levels in term fetuses and were unaffected by labor and delivery. Age of the fetus, determined by the induced ovulation technique (1, 7, 8), correlated inversely with CFI activity in the fetus. This correlation accounted for 93.3% of the change in CFI activity, strongly suggesting that higher CFI activity is a function of fetal immaturity. When these results are applied to humans, CFI levels in the term neonates (14) are equivalent to those in the 127-day-old lamb fetuses.

The reciprocal relationship between CFI and chemotactic activity in sheep and human plasma (13, 14) clearly underlines the critical role CFI may play in the regulation of the inflammatory response. High CFI levels may play a role in the susceptibility of neonates to infections; however, other studies have demonstrated defects in neonatal host defenses at both the cellular and humoral levels. These studies, critically reviewed recently by M. E. Miller (11), have demonstrated a defect in the movement and perhaps the phagocytic function of neonatal neutrophils. Movement of neonatal monocytes may also be deficient. At the humoral level, a decrease in bactericidal and opsonic activities against selected organisms was also demonstrated. Thus, the role of CFI levels in the susceptibility of neonates to infections is yet to be defined. Furthermore, the mechanisms whereby CFI is increased in both mother and infant, and the effects of such risk factors as prematurity, fetal distress and premature rupture of membranes remain obscure. The chronically catheterized fetal lamb model can be used for such studies.

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