Growth factors in milk: interrelationships with somatic cell count

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SUMMARY. Growth factors are thought to play a decisive role in the course of inflammatory processes. The aim of the present study was to characterize a potential interrelationship between the concentrations of insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF) and somatic cell count (SCC) in normal milk, and to investigate the presence of these growth factors in mammary secretions of cows suffering from clinical and subclinical mastitis. Quarter secretions of cows with spontaneous acute clinical mastitis and of cows with subclinical mastitis were analysed radioimmunologically for their concentrations of IGF-1 and bFGF. During two relocation trials with normally lactating Brown Swiss cows, dramatic changes in milk somatic cell count were obtained following a short-term change (5 d) of location and housing system. The animals were relocated from their familiar loose housing system with concrete slatted floor to a separate stanchion barn with long stalls and straw bedding, and *vice versa*. The concentration profile of IGF-1, but not of bFGF, corresponded well with SCC during the relocation trials, the positive correlation between the characteristics being highly significant, as determined by regression analysis ($r = 0{\cdot}60$; $P < 0{\cdot}001$). The results provide evidence that significant changes in SCC and growth factor content may be caused by environmental factors other than infection. The concentrations of both IGF-1 and bFGF were greatly elevated in secretions of quarters affected by acute clinical mastitis compared with the corresponding clinically healthy quarters. Subclinically affected quarters with high SCC, as compared with non-affected quarters with low SCC, also had elevated milk IGF-1, but unchanged bFGF. Measuring of growth factor profiles in milk may have value in the near future in monitoring the state of udder health in addition to SCC.

The introduction of the quota system by the European Union led to an overall decrease in the somatic cell count (SCC) of dairy herds within a few years (Schukken *et al.* 1990). With threshold levels being lowered even further, however, a striking discrepancy is arising between normal fluctuations of SCC within a separate herd, which may be due to physiological conditions, and the reliability of SCC as an indicator of udder health.

During the last few years, basic knowledge has accumulated on the initial phases of inflammation. It is generally accepted that, at the site of inflammation, the cellular immune system may reinforce the inflammatory process by means of a cascade of molecular events. Macrophages, which under normal conditions represent the majority of somatic cells in bovine milk (Lee *et al.* 1980; Fox *et al.* 1985; Holmberg & Concha, 1985), are considered to be the initiators of infectious inflammation in the udder (Kehrli & Shuster, 1994). In response to activation by

bacterial toxins or phagocytosis (Bazzoni *et al.* 1991; Lloyd & Oppenheim, 1992), macrophages are capable of releasing a number of inflammatory cytokines, such as tumour necrosis factor α and interleukin-1, which initiate the general symptoms of inflammation and lead to a massive influx of polymorphonuclear leucocytes (PMNL) into the mammary gland and milk, thus causing an increase in SCC (Kehrli & Shuster, 1994). Following the initial events of inflammation, additional growth factors are involved in tissue regeneration and wound healing processes, for example insulin-like growth factor-1 (IGF-1) (Bird & Tyler, 1994) and basic fibroblast growth factor (bFGF) (Haimovitz-Friedman *et al.* 1991; Vlodavsky *et al.* 1991). Both factors are present in normal mid-lactation milk of healthy quarters, with the concentration of IGF-1 ranging from 10 to 40 (Schams & Einspanier, 1991), and that of bFGF from 0·5 to 1 ng/ml quarter fore milk (Schams, 1994).

In our study, we assessed the potential interrelationships between growth factors, normal milk SCC, and environmental (non-infectious) influences by subjecting a group of cows to a simple modification of their environment in the form of a short-term change of location and housing systems. In addition, in a preliminary study we investigated the concentration profiles of IGF-1 and bFGF in mammary secretions of cows during acute clinical and subclinical mastitis.

materials and methods

Relocation trials

Brown Swiss cows from the Institute's herd in their second to fourth lactation and in their first to tenth month of lactation were assigned to two successive trials.

For the first trial, in June 1994, four animals with chronically elevated SCC in at least one quarter due to a history of mastitis or trauma were taken from their usual loose housing environment (concrete slatted floor, cubicles bedded with chopped straw, twice daily milkings at 05.00 and 16.00 in a 2×2 tandem milking parlour with a BioMilker (BioMelkTechnik Swiss, CH-9053 Teufen 2, Switzerland) at 40 kPa; total mixed rations of maize silage, grass silage and hay with individual portions of concentrate). They were transported to a nearby separate stanchion barn (animals tied in long stalls with straw bedding, rations as usual, milking twice daily with a bucket milking system, BioMilker, 40 kPa) for a period of 5 d and then transferred back to their usual environment. The periods of 4–5 d before relocation and after re-relocation were referred to as control phases. We assumed that previously traumatized animals had a higher susceptibility to environmental factors.

Fore milk samples from each quarter were taken daily at the morning milking for SCC determination and growth factor analysis. Samples for growth factor analysis were immediately defatted by centrifugation at 6000 ***g*** and 4 °C for 30 min and stored at −20 °C until assayed. SCC was determined fluorometrically with a Fossomatic by Milchprüfring Bayern (D-93055 Regensburg, Germany).

The objective of the second experiment, conducted 4 months later, was to verify the results of the first experiment by applying the same experimental design to eight randomly selected animals.

Clinical and subclinical mastitis

Quarter secretions ($n = 48$) from 12 cows affected by clinical mastitis were investigated. The diagnosis of clinical mastitis was based on obvious clinical symptoms of inflammation in the respective quarter, such as rubor, markedly increased temperature of the udder skin, oedema and increased sensitivity to

physical contact. In all cases, the visual appearance of affected quarter secretions differed substantially from that of normal secretions. SCC was not determined, since elevated somatic cell counts can be presumed in clinical mastitis (International Dairy Federation, 1981; Harmon, 1994). Some of the samples were taken from spontaneously occurring clinical cases in the experimental herd. In addition to these, similar samples from cows of the German Fleckvieh breed were kindly provided by Dr Frahm of the Oberschleißheim experimental station affiliated with the Ludwig-Maximilian-University of Munich.

Quarter samples ($n = 88$) from 22 cows (German Fleckvieh) affected by subclinical mastitis were obtained from four small Bavarian farms with chronically elevated bulk tank milk SCC. Affected animals were screened by indirect estimation of quarter SCC (California mastitis test) by Tiergesundheitsdienst Bayern (D-85586 Grub, Germany), and quarter secretions were sampled from animals with a strong positive reaction in at least one quarter.

Growth factor analyses

IGF-1 was determined in skimmed milk samples using an extraction radioimmunoassay technique as described previously (Einspanier & Schams, 1991). Recombinant IGF-1 (Pepro Tech Inc., Tebu, D-60596 Frankfurt, Germany) was used for iodination and as a standard reference. Intra-assay and interassay CV were 3·8 and 16 % respectively.

bFGF was determined by a direct radioimmunoassay technique as described previously (Schams *et al*. 1994). Recombinant bFGF (Pepro) was used for iodination and as a standard reference. The test was validated for skimmed milk samples using dilution curves and recovery experiments. Intra-assay and interassay CV were 4·6 and 9·4 % respectively.

Statistical analyses

Statistical analyses on the two relocation trials were performed for merged data sets employing the SAS program package (SAS, 1992). In order to optimize the normal distribution of the values, the SCC and IGF-1 concentrations for the relocation trials were transformed into their common logarithms. Analysis of variance was then performed using the General Linear Model (GLM) procedure. The differences between the least square means of the experimental phases were tested for significance by means of the Bonferroni–Holm test. The interrelationships between SCC and IGF-1 values were estimated by determination of the Pearson correlation coefficient of the common logarithms. Regression analysis was performed according to the least square method. For results from clinical and subclinical mastitis, only arithmetic means and SD are given. Owing to the small number of animals these latter results can only represent tendencies.

RESULTS

Acute clinical mastitis

Samples from affected quarters had elevated concentrations of IGF-1, ~ 2-fold those for the non-affected quarters of the udder. The concentrations of bFGF in these quarters were 4–6-fold basal levels (Table 1). During the course of acute clinical mastitis in one case (results not shown), a marked increase was observed in the concentration of bFGF in samples from the affected quarter (4·2 *v.* 0·6 ng/ml) 1 d after manifestation of the first clinical symptoms. Levels returned virtually to initial levels over 4 d following veterinary treatment.

Table 1. *Concentrations of insulin-like growth factor-1 and basic fibroblast growth factor in secretions of quarters of Brown Swiss cows affected by acute or subclinical mastitis, and their corresponding healthy quarters*

(Values are ng/ml, means ± SD)

	Quarters affected by acute clinical mastitis	Corresponding non-affected quarters	Quarters affected by chronic subclinical mastitis	Corresponding non-affected quarters
No. of quarters	12	36	28	60
Insulin-like growth factor-1	35·5 ± 23·5	21·2 ± 6·8	36·9 ± 31·3	17·7 ± 11·3
Basic fibroblast growth factor	1·9 ± 0·8	0·7 ± 0·3	0·7 ± 0·3	0·4 ± 0·1

Table 2. *Changes in somatic cell count and concentrations of insulin-like growth factor-1 and basic fibroblast growth factor in quarter samples from Brown Swiss cows relocated from their familiar loose housing to unfamiliar tied housing and* vice versa

(Values are means ± SD)

	Expt no. 1 Housing		Expt no. 2 Housing	
	Loose	Tied	Loose	Tied	Loose
Log(insulin-like growth factor-1, ng/ml)	1·18 ± 0·26	1·03 ± 0·33***	1·15 ± 0·24	0·92 ± 0·33	0·82 ± 0·18***	0·93 ± 0·33
Log(somatic cell count $\times 10^{-3}$/ml)	2·76 ± 0·43	2·20 ± 0·43***	2·72 ± 0·43	2·16 ± 0·73	1·79 ± 0·57***	2·20 ± 0·77
Basic fibroblast growth factor, ng/ml	0·27 ± 0·06	0·27 ± 0·06	0·27 ± 0·05	0·35 ± 0·11	0·39 ± 0·10	0·34 ± 0·08

Values were significantly different from those for loose housing: *** $P < 0{\cdot}001$.

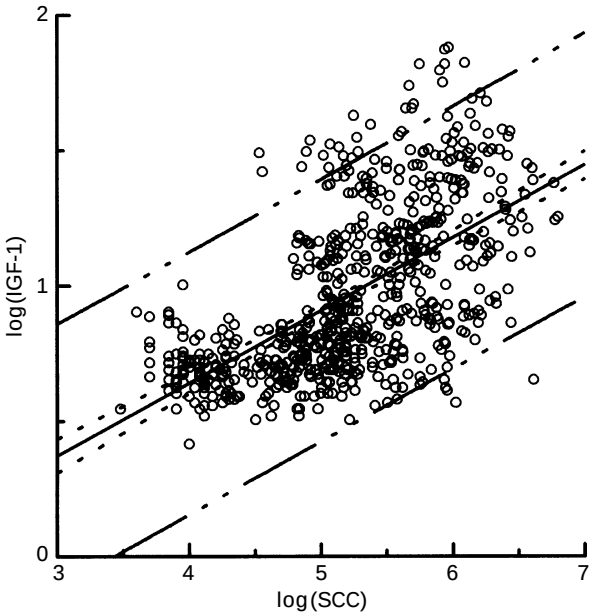

Fig. 1. Linear regression for the relationship between insulin-like growth factor-1 (IGF-1) and somatic cell count (SCC) using merged data sets ($n = 672$) of two relocation experiments with 95% confidence and prediction intervals ($\log(\text{IGF-1}) = 0{\cdot}37 + 0{\cdot}27 \times \log(\text{SCC})$, $r = 0{\cdot}60$, $P < 0{\cdot}001$). Lactating Brown Swiss cows were relocated from their familiar housing system to unfamiliar surroundings, and vice versa.

Subclinical mastitis

Quarters with high SCC (indirect measure) had greatly elevated IGF-1 concentrations in their secretions compared with non-affected quarters, the elevation being comparable to the concentration level present during acute clinical mastitis. The bFGF concentration in secretions of affected quarters was also elevated. However, it barely reached concentrations of > 1 ng/ml, i.e. those characteristic of the acute clinical cases recorded (Table 1).

Interrelationships between SCC and growth factor concentrations in quarter fore milk

During both the relocation trials, relocation and change of housing systems resulted in a uniform, abrupt, highly significant ($P < 0{\cdot}001$) and reversible depression in quarter fore milk SCC (Table 2). Changes in IGF-1 in these samples corresponded with those in SCC, showing a similar depression in concentration during the relocation phase ($P < 0{\cdot}001$, Table 2). bFGF concentrations remained at basal levels throughout both experiments, and there was no correlation with SCC (Table 2). The relations between SCC, IGF-1 and bFGF, estimated for the differences in phase least square means for the entire data set, reflected the conditions on the single quarter level. However, changes in absolute cell counts and IGF-1 concentrations were more pronounced in the quarters of previously traumatized animals with high initial levels.

The positive correlation between SCC and IGF-1 concentration was highly significant ($r = 0{\cdot}60$, $P < 0{\cdot}001$). The linear regression between SCC and IGF-1 concentration is shown in Fig. 1.

discussion

To our knowledge, this is the first evidence of a direct (non-infectious) interrelationship between SCC and the concentration of a given growth factor (IGF-1) in milk. As discussed in a previous paper (Liebe *et al*. 1996), the effect of relocation on SCC was linked neither to immunosuppressive levels of plasma cortisol concentration nor to any influence of bacteriological status, season, age, stage of lactation or nutrition. The interpretation of the results was that significant changes in SCC may be caused by environmental factors other than infection. Disturbing effects of seasonal change, infection status, milking technique or peripheral cortisol concentrations can therefore be excluded. We suggest that the positive correlation of SCC and IGF-1 and the situation during subclinical mastitis (see above) are based on the same immunological events, since IGF-1 has been shown to act in an immunomodulatory manner on leucocytes, even in the cow (for review, see Arkins *et al*. 1993). However, the origin of the IGF-1 found in milk has not yet been elucidated. IGF-1 has been localized in stromal and alveolar cells of the bovine mammary gland (Schams *et al*. 1995), and IGF-1 and some of its binding proteins (IGFBP) are expressed and secreted by the bovine mammary gland *in vitro*. Alveolar epithelial cells secrete both IGF-1 and IGFBP, but only show expression of IGFBP (Campbell *et al*. 1991). Studies at our laboratory revealed that IGF-1 expression in the mammary gland could be detected only during involution, and not during other functional stages (A. Plath & D. Schams, unpublished results).

It has been postulated that IGF-1 is secreted predominantly by macrophages during the initial phases of a wound healing process (Bird & Tyler, 1994). There is evidence that an IGF-1-like molecule is synthesized in the human by activated macrophages (Rom *et al*. 1988; Baxter *et al*. 1991). This molecule is secreted by human macrophage-like cells after stimulation (Nagaoka *et al*. 1990). These findings imply that bovine milk macrophages might secrete IGF-1 directly into milk.

The dramatic and abrupt changes in the IGF-1 concentrations during the relocation trials, however, suggest that most of the IGF-1 measured in milk originates from the circulatory system, and is transferred to the milk through either the activation and deactivation of transport mechanisms or the opening and closing of diffusion barriers. Possible IGF-1 transfer mechanisms to milk have been discussed (Grosvenor *et al*. 1993), and there is evidence that most of the milkborne IGF-1 in the goat may be derived from blood (Prosser *et al*. 1991).

The close relationship between IGF-1 and SCC suggests that IGF-1 might be a crucial factor for the recruitment of somatic cells into milk. IGF-1 has been shown to exert chemotactic attraction on PMNL and T lymphocytes (Tapson *et al*. 1988; Arkins *et al*. 1993), and specific IGF-1 receptors have been demonstrated on the surface of circulating bovine monocytes and PMNL (Zhao *et al*. 1992).

In the present study, elevated IGF-1 and bFGF levels were detected in the secretions of quarters affected by clinical mastitis. An increase of IGF-1 in milk was also observed after an intramammary challenge with *Escherichia coli* (Shuster *et al*. 1995) or with endotoxin (Bruckmaier *et al*. 1993). Furthermore, we have found elevated bFGF concentrations in early post partum secretions (A. Liebe & D. Schams, unpublished results). It has long been known that IGF-1 concentrations are highest prepartum and early post partum (Malven *et al*. 1987; Campbell & Baumrucker, 1989; Prosser *et al*. 1989; Schams & Einspanier, 1991; Prosser, 1996), suggesting that IGF-1 may play an important role in the development of the mammary gland and even of the gastrointestinal tract of the newborn (for review, see

Prosser, 1996). High concentrations of growth factors in post partum secretions have been postulated to participate in establishing normal secretory function (Iivanainen *et al*. 1992). The present results therefore suggest that IGF-1 and bFGF might be important for tissue regeneration processes under acute inflammatory conditions in the mammary gland.

In contrast to the situation during acute clinical mastitis, quarter samples from subclinically affected quarters had increased IGF-1 concentrations, but bFGF was unchanged. This indicates that bFGF might be useful as a distinct marker of severe lesions of the mammary tissue in acute cases of mastitis, as the distribution of bFGF in bovine mammary tissue is restricted to vascular cells and myoepithelial cells during lactogenesis and lactation (Schams *et al*. 1995). In a recent study at our laboratory, a reduced expression of bFGF and FGF receptor in the mammary glands of primiparous heifers was detected during lactogenesis and galactopoiesis, whereas the concentration of bFGF protein in mammary tissue was highest during involution, subsequently falling to a minimum during lactation (Schams *et al*. 1997).

In conclusion, the present study has demonstrated that the SCC of dairy cows seems to be subject not only to physiological and infectious conditions, but also to environmental factors such as housing systems. A change in housing systems can cause dramatic, abrupt and reversible reactions of SCC. Under these conditions, only IGF-1 was strongly positively correlated with SCC.

The interrelationships demonstrated between IGF-1, bFGF and SCC or clinical or subclinical mastitis indicated that growth factor profiles (especially others than IGF-1) may be useful in monitoring the state of udder health. This might also offer an opportunity to redefine common SCC threshold levels for the diagnosis of subclinical mastitis by taking into consideration the physiological or environmental conditions to which an individual animal or herd is subject.

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