Maternal distress and social support are linked to human milk immune properties

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Abstract

Possible alterations of maternal immune function due to psychological stress may reflect immunoactive factor levels in breast milk. This study aimed to assess the association between maternal distress and breast milk levels of secretory IgA (SIgA), IgM, IgG, and lactoferrin (LF). We hypothesized this association is moderated by maternal social support achieved from others during lactation.

The study group included 103 lactating mothers and their healthy 5-months-old infants. Maternal distress was determined based on the State Anxiety Inventory and the level of salivary cortisol. Social support was assessed using Berlin Social Support Scales. Breast milk samples were collected to test for SIgA, IgM, IgG, and LF using the ELISA method. Milk immunoactive factors were regressed against maternal anxiety, social support, salivary cortisol, and infant gestational age using the general regression model.

Maternal anxiety was negatively associated with milk levels of LF ($\beta=-0.22, p<0.05$) and SIgA ($\beta=-0.29, p<0.01$), while social support was positively associated with milk IgG ($\beta=0.25, p<0.05$). Neither anxiety nor social support was related to milk IgM. No association was found between the level of maternal salivary cortisol and immunoactive factors in milk. Our results suggest that maternal psychological well-being and social support may affect milk immune properties.

Keywords: breast milk immunoactive factors, cortisol, maternal stress, social support
Introduction

Maternal milk, defined as perfect food, is the best way of feeding newborns and infants during the first six months of life [1-2]. Besides nutritional value, human milk is rich in immunological factors, including immunoglobulins, lactoferrin, and lysozyme, crucial for newborn and infant immature immune system [3-5]. Antibodies are key factors of an adaptive immune response. Immunoglobulin G (IgG) is the primary antibody class found in the circulation [6]. However, in extracellular fluids, such as maternal milk, the secretory immunoglobulin A predominates [4].

During pregnancy, of all immunoglobulins, IgG is the only antibody class that is transferred from the maternal circulation to the developing fetus across the placenta. The passive transfer of IgG is mediated by the FcRn receptor in a gestational age-related manner [7-8]. After birth, newborns are deficient in functional plasma cells capable of IgG production, but in the first days of life, they can still use the antibodies provided during fetal life. For this reason, milk immunoglobulins, including primary secretory immunoglobulin A (SIgA), immunoglobulin M (IgM), and IgG transferred during breastfeeding, are essential for offspring during the first three months of life [9-10].

The specificity of milk immunoglobulins is determined by different pathogens with which the mother had contact before and during pregnancy [11]. In line with that, the health status of pregnant mothers shapes the immunological properties of their milk. During the first stage of lactogenesis, an increase in lactose, protein, and immunoglobulin levels is observed. Since milk production begins already at 28 weeks of normal pregnancy [12], perinatal risk factors have a substantial impact on the onset and duration of lactation as well as on the nutritional and protective value of maternal milk [13-14].

Psychological stress is a constant element of our lives that affects body function through the nervous system's direct action and the network of secreted hormones [15]. The impact of stress, including psychological stress, on immunity is well established [reviewed 16]. The evoked immunological response can be protective, pathological, or regulatory depending on its intensity, duration, and chronicity [17]. On the other hand, scientific data also indicates that the immune system impacts stress resistance and coping with stress [18]. Psychological stress modulates the immunological system at different levels- from regulating single immunological factors to the whole system and ultimately affecting physical health [16].

Pregnancy and perinatal period, including successful breastfeeding, are stages of increased exposure to stress associated with the physiological course and significant energy costs of pregnancy, childbirth, and lactation. Adjustment to entirely new conditions and life roles
after birth adds another psychological component of stress, which severity may differ depending on maternal status and social support [19-20]. Since milk serves as an important signal of the maternal physiological state passed to an infant during breastfeeding [21], possible alterations of maternal immune function in response to stress may reflect the levels of immunoactive factors in breast milk [22].

However, evidence for the effect of maternal distress on milk immune properties is mixed and limited mainly to SIgA [23-27] but see [28]. Some of the previous research [23,25,27] conducted in newborns demonstrated an association between milk SIgA and different questionnaire measures of maternal mood and distress. Other studies [24,26,28] demonstrated a lack of the association between levels of milk SIgA or other immunoactive factors (IgG, IgM, interleukins, growth factors and chemokines) and maternal distress assessed either with questionnaires or quantified by measures of cortisol.

The observed discrepancies in the study results come from several sources, including relatively small sample sizes, variable methods to assess maternal distress, and early or varying infant age. These discrepancies call for further systematic studies examining a wider range of milk immunoactive components. Furthermore, other factors with the ability to modify the maternal response to stress, like social support and coping mechanisms, should also be included. They might confound or moderate the association between maternal stress and milk immune properties. A number of previous studies demonstrated that social support is related to better immune function [29], probably via blunting stress responsiveness [30]. Our preliminary results also suggest that maternal support might be associated with the nutritional milk composition [31].

This study aims to further investigate these discrepancies by studying the association between various markers of milk immune properties (levels of SIgA, IgG, IgM, and lactoferrin) and maternal distress characterized by the state of anxiety and reactivity to a stressor measured with salivary cortisol. We studied this association in a sample of women homogenous in terms of education, socioeconomic status, and infant age. Following previous research, we hypothesize that maternal distress would be negatively related to milk immune properties. Furthermore, the study also aims to assess the association of milk immunoactive components with maternal social support. We hypothesize that maternal social support would be positively related to milk immune properties.

Methods

Study sample and protocol
A sample of 160 mothers and their healthy, born on term infants from Wroclaw, South-Western Poland, took part in a study on the association between maternal stress and breast milk composition. Participants were recruited to meet the following inclusion criteria: 1) neither mothers nor infants suffered from metabolic diseases such as diabetes or thyroid diseases and genetically inherited conditions; 2) infants were born from a single, uncomplicated pregnancy, with the appropriate birth weight for gestational age not lower than 2500 g; 3) infants were fed on-demand, exclusively with breast milk. In addition, for the current analysis, all mothers who suffered from any infections (ongoing or during the two weeks prior to the study) were also excluded due to their possible effect on the level of produced immunoactive factors [23]. Thus, the final number of participants was reduced to 103.

During the first meeting scheduled when the children were approximately five months old, the mothers signed the informed consent and were instructed about all study procedures. Trained study assistants performed maternal anthropometric measurements. Mothers also received a general questionnaire to be filled in at home about basic maternal demographics such as age, birth, education, life, economic satisfaction, marital status, reproductive history, health, and basic infant characteristics. They also received psychological questionnaires to assess anxiety and social support. These questionnaires were returned at the second meeting approximately one week later. At this meeting, a single milk sample was taken and the cold-pressor test [32] was conducted to assess maternal hormonal response to a mild stressor. The study protocol was approved by the Bioethical Committee of Lower Silesian Medical Chamber in Wroclaw.

Maternal anxiety and social support

Maternal anxiety as a state and trait were assessed using the Polish version of the State-Trait Anxiety Inventory (STAI) [33]. This questionnaire is based on a 4-point Likert scale and includes 40 questions, 20 items for assessing trait anxiety and 20 for state anxiety [34].

Social support, which participants received from others, was assessed using Polish adaptation [35] of the Berlin Social Support Scale (BSSS) [36]. The questionnaire is also based on a 4-point Likert scale. The questionnaire allows for the assessment of instrumental, emotional, and informational support as well as the perceived need for this support. In our analysis, we use the sum of the points given to different support forms with the exclusion of the need for support.

Milk samples
Mothers collected milk samples in sterile containers using a Medela Symphony breast pump (Medela AG, Switzerland) at the meeting room under the research assistants' supervision. Mothers were instructed to pump milk from one breast until empty. To standardize milk collection time against possible diurnal changes in breast milk composition, samples were collected between the second and the third feeding episode of the day, where the first feeding was the one after which an infant showed daytime activity [37-38]. Since no significant difference in milk composition was found between the left and right breast [39], the mothers were free to choose from which breast milk was collected. Immediately after collection, milk samples were stirred and portioned into smaller containers and stored at -80°C for later analysis.

Determination of immunoglobulin and lactoferrin concentration in milk

Prior to determining immunoglobulin and lactoferrin concentrations in milk, all collected milk samples were centrifuged at 3 500 g at 4°C for 35 minutes to obtain defatted milk samples. The aqueous phase of analyzed milk samples was stored at -20 °C.

The concentrations of all analyzed immunoglobulins, namely, SIgA, IgG, IgM, and lactoferrin (LF) in defatted milk (skim milk) samples, were quantified by the enzyme-linked immunosorbent assay (ELISA) using a previously developed procedure with modification [40-42]. In short, microtiter plates (Nunc International, Naperville, IL, USA) were used to determine all immunoglobulins and LF levels. For blocking and washing steps, TBS (pH 7.5) containing 0.5% Tween-20 and TBS (pH 7.5) containing 0.05% Tween-20 were used, respectively. The antibodies applied in the tests were: for IgG F(ab')2 fragments of goat anti-human IgG (Jackson ImmunoResearch, Europe Ltd., Ely, UK), for IgM rabbit anti-human IgM antibody (Jackson ImmunoResearch, Europe Ltd., Ely, UK), for SIgA mouse monoclonal anti-secretory component IgA antibodies (Sigma, St. Louis, MO, USA) and for lactoferrin rabbit anti-human lactoferrin antibodies phosphatase-labeled (Jackson ImmunoResearch Europe Ltd., Ely, UK). Standard curves were constructed using commercially available preparation of human serum IgG (Jackson ImmunoResearch, Europe Ltd., Ely, UK), human IgM (Jackson ImmunoResearch, Europe Ltd., Ely, UK), human colostrum IgA (Sigma, St. Louis, MO, USA), and human milk lactoferrin (Sigma Aldrich, St. Louis, MO, USA), respectively. For detection, horseradish peroxidase (HRP) and alkaline phosphatase (AP) labeled antibodies were used, namely, for IgG phosphatase-labeled rabbit anti-human IgG Fcγ fragment-specific antibodies (Jackson ImmunoResearch, USA), for IgM horseradish peroxidase-conjugated goat anti-human IgM antibodies (Jackson ImmunoResearch, Europe Ltd., Ely, UK), for S-IgA horseradish peroxidase-conjugated goat anti-mouse IgG antibodies (Sigma, St. Louis, MO, USA), and for LF
rabbit anti-human lactoferrin antibodies phosphatase-labeled (Jackson ImmunoResearch Europe Ltd., Ely, UK). The enzymatic reactions for HRP and AP were developed with appropriate substrates. The obtained absorbances were quantified at 492 nm (reference filter 630 nm) for HRP and at 405 nm (reference filter 630 nm) for AP, using a Stat Fax 2100 Microplate Reader (Awareness Technology Inc., Palm City, FL, USA).

All defatted milk samples were quantified at three different sample dilutions dedicated to the individual parameter, each in duplicate. The calculated intra-assay and inter-assay coefficients of variation ranged from 3.2% to 4.8%, for IgM determination from 4.1 to 3.6%, for SIgA determination from 2.8 to 3.3%, and for LF determination from 4.3% to 3.6%, respectively.

Salivary samples

The hand cold pressor test (CPT) [32] was performed to assess maternal physiological reactivity to a mild stressor. Women were asked to immerse the hand into ice water for one minute. Four saliva samples to measure cortisol level (Csal) were taken 1) 10 min. before; 2) a min. before; 3) immediately after, and 4) 10 min. after the test. The average level of Csal measured in all saliva samples was calculated to include in further analysis.

Cortisol in saliva

Samples were collected into sterile 1 ml Eppendorf tubes and stored at -80°C until the assay. After thawing and centrifugation (1500g for 10 min), samples were tested for salivary C concentration using enzyme-linked immunosorbent assays (Salivary Cortisol ELISA, DRG Instruments GmbH, Germany) according to the manufacturers' recommendations. The samples were analyzed in duplicate, and the average intra-assay coefficient of variation for cortisol was less than 4.5%.

Statistical analysis

The distribution of milk immunoglobulins (SIgA, IgM, IgG) and salivary cortisol (Csal) diverted from normal; thus, all their values were log-transformed to assure normality. Possible differences between primiparous and multiparous women in levels of immunoactive factors, infant age, and gestational age were tested with Student t-test. Linear associations between variables were tested using Pearson correlations.

The association between maternal anxiety, social support, and milk's immunological properties was analyzed using the general regression model. Each immunoactive factor (LF, SIgA, IgM,
IgG) was regressed against maternal anxiety, logCsal, and social support together with gestational age as a possible confounder in multiple separate models. For each model, collinearity was tested using the Durbin-Watson test for autocorrelation. All statistical analysis was performed using Jamovi, version 1.6 (2020) [43].

Results

Out of 103 women (mean age 31.1 ± 4.09) included in the analyses, almost 60% were primiparous; thus, mean parity was 1.5 ± 0.65. Most women (over 80%) had higher education, confirmed by at least a bachelor's degree. All women lived with their partners or husbands, were non-smokers, and did not drink alcohol.

No statistical differences in maternal and infant anthropometrics were found between the primiparous and multiparous women. Multiparous women were significantly older (t(102)=−3.95, p<0.01) and had lower levels of milk IgM than primiparous women (t(102)=3.60, p<0.01). No other differences in milk immunoactive factors between groups were found. Multiparous women suffered from higher anxiety (t(102)=−2.21, p<0.05) and received lower social support (t(102)=4.10, p<0.01) but had similar to primiparous women levels of salivary cortisol. General characteristics of the total sample and primiparous and multiparous women are listed in Table 1.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>All (n=103)</th>
<th>Primiparous (n=61)</th>
<th>Multiparous (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mothers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>31.1 (0.40)</td>
<td>29.7 (0.47)*</td>
<td>32.9 (0.62)*</td>
</tr>
<tr>
<td>Education (% higher)</td>
<td>84.5%</td>
<td>83.8%</td>
<td>81.0%</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.0 (0.34)</td>
<td>23.5 (0.46)</td>
<td>22.4 (0.49)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>36.1 (0.67)</td>
<td>35.0 (0.91)*</td>
<td>38.0 (0.94)*</td>
</tr>
<tr>
<td>Social support</td>
<td>47.3 (0.57)</td>
<td>49.2 (0.62)*</td>
<td>44.8 (0.93)*</td>
</tr>
<tr>
<td>Csal (ug/dL)</td>
<td>1.93 (0.155)</td>
<td>2.04 (0.185)</td>
<td>1.78 (0.275)</td>
</tr>
</tbody>
</table>

Infants (57 boys, 46 girls)

| Age (months) | 4.8 (0.05) | 4.7 (0.07) | 4.8 (0.08) |
Gestational age (weeks)  39.8 (0.14)  40.0 (0.18)  39.5 (0.20)
Birth weight (kg)  3.5 (0.04)  3.5 (0.06)  3.6 (0.06)
Birth length (cm)  54.7 (0.27)  54.9 (0.36)  54.3 (0.41)

**Breast milk**

<p>| | | | |</p>
<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>Lactoferrin (g/L)</td>
<td>3.28 (0.100)</td>
<td>3.20 (0.137)</td>
<td>3.4 (0.145)</td>
</tr>
<tr>
<td>SIgA (g/L)</td>
<td>1.62 (0.045)</td>
<td>1.59 (0.050)</td>
<td>1.68 (0.083)</td>
</tr>
<tr>
<td>IgG (mg/L)</td>
<td>11.7 (0.396)</td>
<td>11.86 (0.530)</td>
<td>11.57 (0.602)</td>
</tr>
<tr>
<td>IgM (mg/L)</td>
<td>2.38 (0.167)</td>
<td>2.68 (0.226)*</td>
<td>1.29 (0.206)*</td>
</tr>
</tbody>
</table>

* p<0.05

Tab. 1 General characteristics of all study participants and differences in general characteristics between primiparous and multiparous women.

No significant correlation was found between maternal age, life and economic satisfaction, and levels of milk immunoactive factors. In contrast, a significant correlation was found for infant gestational age. Gestational age correlated negatively with lactoferrin (r=-0.30, p<0.01) and SIgA (r=-0.20, p<0.05). Maternal level of anxiety correlated negatively with the level of social support (r=-0.38, p<0.001), while neither anxiety (r=-0.11, p=0.28) nor support (r=0.03, p=0.76) correlated with the level of CsAl.

**Relationship between maternal distress, social support, and milk immunoactive factors**

Maternal distress and social support were significantly associated with milk immunoactive factors (Table 2, Figure 1). In particular, the level of lactoferrin was negatively related to maternal anxiety ($\beta$=-0.22, p<0.05), but no association was found with social support ($\beta$=-0.03, p=0.73). Another significant factor negatively predicting the level of lactoferrin was infant gestational age ($\beta$=-0.29, p<0.01). The whole model, which included postanal age and salivary cortisol levels, explained around 11% of the variance in the level of lactoferrin.
Fig. 1 The association between maternal state of anxiety and social support, and the level of immunoactive components in human milk. Negative association found for the level of lactoferrin (A) and SIgA (B) and maternal anxiety. Positive association found for the level of IgG and social support (C).
The level of SIgA in milk was negatively related to maternal anxiety ($\beta = -0.29$, $p<0.01$), but no significant association was found for social support ($\beta = -0.19$, $p>0.05$). Another factor that predicted the level of SIgA was infant gestational age; however, this association was marginally significant ($\beta = -0.19$, $p=0.05$). All factors included in the model together explained 10% of the variance in the level of SIgA.

The level of IgG in milk was positively related only to social support ($\beta = 0.25$, $p<0.05$), while no association was found for maternal anxiety ($\beta = 0.01$, $p=0.92$). The level of IgG was also positively related to postnatal infant age ($\beta = 0.26$, $p<0.05$). All factors included in the model explained around 9% of the variance in IgG level.

In contrast, neither maternal anxiety ($\beta = -0.18$, $p=0.10$) nor social support ($\beta = 0.12$, $p=0.27$) predicted IgM’s level in milk. The whole model, which included gestational, postnatal age, and salivary cortisol level, was statistically not significant. Table 2 presents the detailed results of the regression models.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>$\beta$</th>
<th>95% CI for $\beta$</th>
<th>$p$</th>
</tr>
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<tbody>
<tr>
<td>LF ($F(5,97)=3.58$, $p&lt;0.01$, $R^2_{adj}=0.11$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>-0.22</td>
<td>-0.425 - -0.017</td>
<td>0.034</td>
</tr>
<tr>
<td>Social support</td>
<td>-0.03</td>
<td>-0.239 - 0.168</td>
<td>0.731</td>
</tr>
<tr>
<td>logCsal</td>
<td>-0.09</td>
<td>-0.286 - 0.096</td>
<td>0.330</td>
</tr>
<tr>
<td>Infant age</td>
<td>0.11</td>
<td>-0.080 - 0.306</td>
<td>0.249</td>
</tr>
<tr>
<td>Gestational age</td>
<td>-0.29</td>
<td>-0.479 - -0.104</td>
<td>0.003</td>
</tr>
<tr>
<td>log SIgA ($F(5,97)=3.38$, $p&lt;0.01$, $R^2_{adj}=0.10$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>-0.29</td>
<td>-0.501 - -0.089</td>
<td>0.005</td>
</tr>
<tr>
<td>Social support</td>
<td>-0.20</td>
<td>-0.402 - 0.008</td>
<td>0.060</td>
</tr>
<tr>
<td>logCsal</td>
<td>0.11</td>
<td>-0.077 - 0.308</td>
<td>0.238</td>
</tr>
<tr>
<td>Infant age</td>
<td>0.11</td>
<td>-0.088 - 0.300</td>
<td>0.249</td>
</tr>
<tr>
<td>Gestational age</td>
<td>-0.19</td>
<td>-0.375 - 0.002</td>
<td>0.053</td>
</tr>
<tr>
<td>log IgG ($F(5,97)=2.86$, $p&lt;0.02$, $R^2_{adj}=0.08$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>0.01</td>
<td>-0.196 - 0.219</td>
<td>0.916</td>
</tr>
<tr>
<td>Social support</td>
<td>0.25</td>
<td>0.043 - 0.457</td>
<td>0.018</td>
</tr>
<tr>
<td>logCsal</td>
<td>0.14</td>
<td>-0.049 - 0.339</td>
<td>0.145</td>
</tr>
</tbody>
</table>
Tab. 2 Results of the regression models for the association between maternal distress (anxiety, logCsal), social support and levels of milk LF, SIgA, IgG and IgM

Discussion
This study presents evidence for the effect of maternal distress and social support on breast milk immunoactive properties. In particular, it demonstrates a positive association between the mother's social support and the level of IgG in her milk. Furthermore, it supports the previously described association between maternal anxiety and levels of milk SIgA, but also shows that anxiety may be linked to the level of other immunoactive factors such as milk lactoferrin.

To our knowledge, this is the first study demonstrating that maternal social support is associated with immunoactive compounds of breast milk. Previous research showed that higher support from others was associated with earlier breastfeeding initiation and a longer period of total breastfeeding [44-48]. Our preliminary results also show that the number of people who helped lactating mothers was positively related to the content of polyunsaturated fatty acids (PUFA) in milk [31]. Physiological mechanisms beyond this effect might include the reduction of chronic psychosocial stress associated with motherhood. The role of long-term and chronic psychosocial stress in impairing immune function has been widely recognized [49]. Several studies demonstrated decreased blood or saliva immunoactive factors in response to chronic stress [50].

On the other hand, studies demonstrated that social support modifies the stress response. In particular, Heinrichs and others (2003) [30] showed that social support decreased cortisol levels in response to TSST (Trier Social Stress Test), an experimental procedure to induce high psychosocial stress. Higher social support was also positively related to the level of antibodies,
including IgG and IgM, after different vaccinations [51]. These results are of particular significance to our study, which showed a positive association between maternal social support and milk IgG levels. Apart from the local synthesis in the mammary gland, the milk level of IgG is supported by the active transfer of IgG from maternal serum [52]. Thus, the higher level of IgG in milk is most probably an indicator of a higher level of IgG in maternal serum. While social support and anxiety were significantly and negatively correlated, a positive association between social support and milk IgG may reflect the common pathway of interaction between stress, social support, and immune function.

Our study found a negative association between the state of anxiety and levels of SIgA, which is the main milk immunoglobulin. This result corroborates some of the previous works demonstrating the negative effect of maternal distress on milk immunoactive properties. In particular, Kawano and Emori (2015) [25] found a similar negative association between maternal anxiety state and milk SIgA in Japanese mothers on the second or third day postpartum. Our results suggest that this effect may extend to the later stages of breastfeeding. Moreover, the level of maternal anxiety observed in this population was similar to ours (mean score 39.6 vs. 36.1).

Interestingly, a large recent study by Aparicio and others (2020) [28] found no effect of maternal psychological distress (including anxiety) on the level of milk SIgA or any other analyzed immunoactive factors. Comparing to our study, this lack of association might result from differences in milk collecting protocol (foremilk samples vs. whole milk samples), lower infant age (2, 6, or 12 weeks vs. 5 months), different geographical location [53], and decreased levels of maternal anxiety (median score 27.0 vs. 36.3). The differences in the observed level of anxiety between the studies may suggest that the negative relationship with SIgA is revealed only after a specific maternal anxiety threshold is reached.

The specificity of transferred milk immunoglobulins is limited to the range of pathogens with which the mother's immune system had contact previously [11, 54]. Milk immunoglobulin composition, especially in a qualitative context, i.e., specificity, might be shaped before and already during pregnancy by the previous infection, vaccination, and mother's allergies [55]. In turn, the overall effect of psychosocial factors on the level of milk immunoglobulins is modest. However, stress-related low-grade inflammation may interfere with the normal function of immune B cells [56] and thus modulate the production of all immunoglobulins, or only a particular class of immunoglobulins.

Results of our analysis also demonstrated a significant negative association between maternal distress and the level of LF. Little is known about the external factors that influence
the level of LF in human milk. Previous studies identified lactational stage [57], maternal age [58], and ethnicity [59] as the main factors regulating variation in the level of milk LF. Our research identifies maternal anxiety as yet another factor. Although no such analysis has been published before, experimental studies in rodents and humans suggest a possible physiological base for this association. Single-dose oral administration of bovine LF was found to mitigate the physiological response to experimentally induced psychological stress in rats [60] and humans [61]. Thus, it is possible that in breastfeeding mothers with increased anxiety, LF is preferentially used to alleviate the consequences of higher stress and LF transfer to milk remains low.

The indisputable strength of the demonstrated results is a highly valuable and well-characterized cohort of mothers with stabilized 5th month lactation. Moreover, a very homogenous cohort in terms of gestational age and residence place eliminates the possible impact of geographical location on the immunological quality of the analyzed milk [53,62]. However, a single sample collection protocol and observational character may limit the study's generalizability to a single time point during lactation. Future studies should also seek to include mothers from the general population with a broader socioeconomic status and education range.

In summary, our study provides evidence for a significant association between the mother's psychological well-being and the immunological properties of her milk. Although evidence from the literature is mixed, the study suggests that increased anxiety, most probably associated with higher psychological stress, negatively affects milk immune properties. In contrast, social support, which was demonstrated to mitigate response to stress and strengthen the individual immunological response to challenge, may also boost milk properties. Noteworthy, both factors interacted with different markers of milk immune properties, which may suggest different physiological pathways of the observer associations. Since maternal well-being is crucial for ensuring the adequate immune protection of their breastfed children, the results of our study advocate intensifying social support of mothers during pregnancy and breastfeeding as an overarching public health goal.

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Author contributions

Institutional Review Board Statement
The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Lower Silesian Medical Chamber in Wroclaw (protocol code 1/NT/2016 from 10.02.2016)

Informed Consent Statement
Informed consent was obtained from all subjects involved in the study.

Data Availability Statement
The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ongoing analysis.

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