POST-PARTUM FLARE IN MRL-\textit{lpr}/\textit{lpr} MICE IS ASSOCIATED WITH A PARALLEL INCREASE OF 
\textit{N}-ACETYLGLUCOSAMINE ON SERUM IgG

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SUMMARY

Our objective was to investigate IgG glycosylation and disease activity post partum in the MRL-\textit{lpr}/\textit{lpr} mouse. Disease activity was monitored using bimalleolar ankle swelling. Levels of galactose and \textit{N}-acetylglucosamine (GlcNAc) on IgG isolated from serum were measured using biotinylated lectins. Our results show that disease severity increased post partum. This post-partum flare correlated significantly with an increase in IgG GlcNAc expression. The disease severity increased post partum in the MRL-\textit{lpr}/\textit{lpr} mouse model similar to the changes seen in rheumatoid arthritis (RA).

KEY WORDS: MRL-\textit{lpr}/\textit{lpr}, Pregnancy, Post partum, Immunoglobulin, Glycosylation.

In 1938, Hench reported that in the presence of pregnancy, rheumatoid arthritis (RA) ‘finds it difficult to progress, or indeed to do otherwise than to beat a rather precipitous retreat’ [1]. This observation has been supported by several studies, showing that in the majority of pregnant patients (73%) symptoms improve. However, \sim 90\% of these patients have a post-partum recurrence [2]. Unlike RA, the past literature on systemic lupus erythematosus (SLE) during pregnancy has been rather confusing. This has been due to the lack of agreement regarding disease activity as well as distinguishing between pregnancy complications such as pre-eclampsia and SLE [3]. Definitions of exacerbation and remission have not been clearly stated, and the timing of such events not recorded. However, Cecere and Persill [4] reviewed the history of pregnancy and the outcome of disease in SLE, and concluded that flare-ups may occur at any time during gestation, but are particularly common post partum. Where there is active disease at conception, exacerbation is more likely during pregnancy in SLE [5].

The mechanism responsible for the effects in these diseases has not yet been established, although a number of immunological and endocrinological explanations have been submitted. One possibility in RA may be the glycosylation status of immunoglobulin G (IgG). The carbohydrate composition of IgG from RA patients shows a decreased galactose content, when compared with that from normal individuals [6], with IgG oligosaccharide chains terminating in \textit{N}-acetylglucosamine (GlcNAc) rather than a galactose/sialic acid residue. In RA, during pregnancy, IgG composition may alter in such a way that it appears ‘normal’. It has been demonstrated in both normal women and arthritic patients that galactose levels rise throughout pregnancy and then fall after delivery [7]. The pathogenic relevance of these oligosaccharide changes, although uncertain, is crucial for the formation of self-associated immune complexes [8, 9].

The MRL-\textit{lpr}/\textit{lpr} mouse spontaneously develops a disease syndrome which has been used as a model for RA and SLE, where the pathological changes in the joints include destruction of articular cartilage, proliferation of synovium and pannus formation [10, 11]. During routine breeding of MRL-\textit{lpr}/\textit{lpr} mice, we noticed that a significant number developed post-partum erythema and swelling, so have used this model to examine the effect of physiological doses of oestradiol on the post-partum flare-up [12]. In view of our previous finding that glycosylation of IgG from the MRL-\textit{lpr}/\textit{lpr} mouse differs from that in normal mice [13], we have now investigated whether the post-partum increase in disease activity, in these inbred mice, is linked with further changes in serum IgG glycosylation.

METHODS

\textit{Animals}

MRL-\textit{lpr}/\textit{lpr} mice were obtained from a breeding colony maintained in the Department of Oral Biology, University of British Columbia, Vancouver, BC, Canada, originally established from stocks purchased from the Jackson Labs (Bar Harbor, ME, USA). Ten female MRL-\textit{lpr}/\textit{lpr} mice were mated and produced litters.

\textit{Clinical evaluation}

Clinical measurements were made on the following days, \sim -7, 0, 5, 10, 15, 25 and 30, with regard to birth. Bimalleolar ankle width measurements were made using a micrometer. Previously, the intra-examiner reliability of the measurement was established as excellent after ranking measurements with the Kendall coefficient of concordance ($\tau = 0.784$, $P < 0.01$). The sensitivity was established at 0.1 mm based on the mean of the range of the intra-examiner measurements.
One animal died (symbol +) after day 10 so there were nine ankle measurements at day 30.

**IgG glycosylation**

Glycosylation analysis was carried out on purified serum IgG using our previously described lectin binding assay [10], where *Ricinus communis* agglutinin I (RCA I) and *Bandeiraea simplicifolia* II (BSII) were used to detect galactose and GlcNAc, respectively. Results are expressed as absorbance units. Each sample was measured in triplicate and mean values plotted. Collection of serial bleeds from pregnant mice was difficult (especially the amount necessary for glycosylation analysis) but, where possible, IgG was purified from serum samples on days −7, 0, 10 and 30. Insufficient serum was obtained to allow glycosylation analyses on a few occasions, but particularly on day 30 (symbol Δ) where eight out of 10 IgG samples were analysed.

**Statistical analysis**

A two-tailed paired Student’s *t*-test was used when analysing differences between time points. Spearman’s rank correlation was used to determine the association between GlcNAc expression and ankle width.

**RESULTS**

**Clinical evaluation**

As an evaluation of swelling, related to arthritis onset, bimalleolar measurements were made of both hindlimbs and calculated as mean ankle width for each animal over time.

Up until day 10, there was very little overall change in the mean ankle width of the group. A total of 8/9 individuals showed a clear post-partum flare between days 10 and 30. This change in mean ankle width between days 10 and 30 was clearly significant

\[ t = -3.76, \ P < 0.003; \text{Fig. 1}. \]

One individual showed a decrease in ankle width between days 10 and 30.

**IgG glycosylation**

*N*-Acetylglucosamine (GlcNAc). In parallel with the post-partum flare, there was a dramatic increase in the mean GlcNAc expression between day 10 and day 30 \( (t = -2.23, \ P < 0.03) \), where the mean ± s.e. at day 30 was 748 ± 89 units compared with 482 ± 46 units at day 10 (Fig. 2). A total of 7/8 individuals showed an increase; one serum sample from day 30 was not available for testing.

In contrast to the change in ankle width between day −7 and day 10, there was a smaller but statistically significant decrease in the mean GlcNAc expression between days −7 and 10 \( (t = 3.47, \ P < 0.004) \).

**Correlation of GlcNAc levels and ankle swelling.**

There was a significant correlation between GlcNAc expression and ankle swelling on day 30 \( (r = 0.64, \ P < 0.043) \), as well as a significant positive correlation between the change in GlcNAc expression and the change in mean ankle width between days 10 and 30 \( (r = 0.71, \ P < 0.023) \).

One animal (symbol Δ) did not flare at day 30, as measured by a decreased ankle swelling, but glycosylation analysis was not possible due to the collection of insufficient blood. However, the animal which showed a decrease in IgG GlcNAc expression between days 10 and 30 was the same individual which had the smallest increase in ankle width between those days (symbol ▲).

**Galactose.** When analysing galactose expression with time, there was little difference between the levels detected at day 30 compared with those detected at day −7 (data not shown).

**DISCUSSION**

In RA, during gestation, the disease often remits, while delivery is frequently followed by a relapse [14].
Our study has shown that MRL-\textit{lpr/lpr} mice have increased disease severity (as measured by ankle swelling) following delivery. The post-partum flare in this spontaneous mouse model correlates significantly with an increase in IgG GlcNAc expression. The findings that the absolute values of GlcNAc expression and ankle swelling correlate during the post-partum flare (day 30), together with the correlation of the degree of change of these two parameters (between days 10 and 30), suggest that GlcNAc expression appears to be closely associated with the development of arthritis. It has been postulated that agalactosylated IgG may be responsible for inducing rheumatoid factor production [6] and immune complex formation, and we have shown that there is significantly more GlcNAc expression in complexes from patients with RA compared with complexes from controls [9]. Agalactosyl IgG is strongly associated with pathogenicity in collagen-induced arthritis, the passive transfer of disease being more effective if the transferred IgG is pre-digested with \(-\)galactosidase (to remove terminal galactose), indicating that altered glycosylation of the autoantibody is crucial for it to have a pathological role [15].

Although we have shown that levels of GlcNAc change with the post-partum flare, the mechanisms for this change remain unknown. Mattsson et al. [16] suggested that post-partum flare-up is due to the surge in prolactin release after delivery. Breastfeeding is associated with a significant increase in the risk of developing RA, where prolactin has been postulated as the pro-inflammatory hormone [17]. In 1982, Berczi and Nagy [18] showed that prolactin is needed for arthritis induced using Freund’s complete adjuvant, indicating that altered glycosylation of the autoantibody is crucial for it to have a pathological role [15].

The lack of apoptosis has been suggested to be the reason behind the continued survival of autoantibody-producing lymphocytes [30]. In particular, the CD23\textsuperscript{pos} population of B cells, which normally show high levels of apoptosis, remain prominent in the \textit{lpr} mice. It is tantalizing to think that this set of B cells may be responsible for both the production of autoantibodies and immunoglobulins with altered glycosylation.

We now need to know (a) whether there are two processes contributing to worsening of the disease (an increase in the level of GlcNAc on IgG together with an increased level of prolactin) or (b) whether the level of prolactin causes a change in GlcNAc levels which enhances the arthritis process.

**References**


