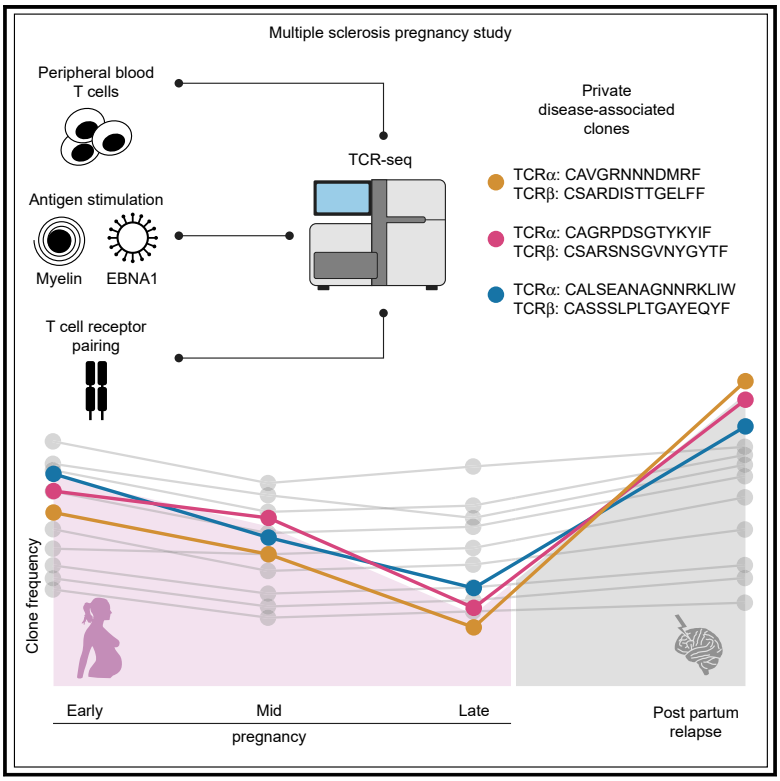
Cell Reports

T Cell Repertoire Dynamics during Pregnancy in Multiple Sclerosis

Graphical Abstract



Highlights

- Pregnancy modulates the T cell repertoire in MS in a clonally specific fashion
- "Private" candidate clones associated with disease activity can be identified
- Barcoding enables α-β TCR pairing of these clones for further characterization
- Most candidate clones do not respond to putative MS autoantigens (e.g., myelin)

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In Brief

Ramien et al. interrogate the immune repertoire in multiple sclerosis (MS) during pregnancy. They report a shift in T cell repertoire composition driven by a small number of "private" clones. This specific rather than global immunomodulation may help to explain the protective effect of pregnancy in human autoimmunity.

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T Cell Repertoire Dynamics during Pregnancy in Multiple Sclerosis

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SUMMARY

Identifying T cell clones associated with human autoimmunity has remained challenging. Intriguingly, many autoimmune diseases, including multiple sclerosis (MS), show strongly diminished activity during pregnancy, providing a unique research paradigm to explore dynamics of immune repertoire changes during active and inactive disease. Here, we characterize immunomodulation at the single-clone level by sequencing the T cell repertoire in healthy women and female MS patients over the course of pregnancy. Clonality is significantly reduced from the first to third trimester in MS patients, indicating that the T cell repertoire becomes less dominated by expanded clones. However, only a few T cell clones are substantially modulated during pregnancy in each patient. Moreover, relapse-associated T cell clones identified in an individual patient contract during pregnancy and expand during a postpartum relapse. Our data provide evidence that profiling the T cell repertoire during pregnancy could serve as a tool to discover and track "private" T cell clones associated with disease activity in autoimmunity.

INTRODUCTION

Identification of relevant T cell clones in complex human autoimmune disorders has remained challenging due to the enormous interindividual variability in repertoire composition and diversity (Hohlfeld et al., 2016a, 2016b). Therefore, cross-sectional case-control studies have limited power and are unlikely to yield informative differences at the group level. Rather, identifying such clones, which are likely "private" (i.e., specific to individual patients), will require longitudinal assessments over periods with rapid changes of disease activity and remission. Pregnancy has a pronounced and clinically relevant impact on maternal autoimmunity, as reflected by a protective effect of pregnancy on disease activity of many autoimmune disorders (Barrett et al., 1999; Confavreux et al., 1998; de Man et al., 2008; Finkelsztejn et al., 2011). In multiple sclerosis (MS), the relapse rate is reduced by 70%–80% in the third trimester (Confavreux et al., 1998; Finkelsztejn et al., 2011), which exceeds the effectiveness of many of the currently available therapies for this disease. Thus, pregnancy could serve as a highly informative research paradigm to uncover relevant T cell clones in individual patients with autoimmune disorders.

Pregnancy is also interesting from an evolutionary perspective, as it poses a challenge to the maternal immune system in placental mammals to balance prevention of immune attacks against the fetus while maintaining immunocompetence of the mother against infections. Strong evolutionary pressure on reproduction has led to highly specific immune adaptation to mammalian pregnancy (Samstein et al., 2012) and can thus offer insights into mechanisms of endogenous immune tolerance.

Many pregnancy hormones have immunomodulatory properties that could exert nonspecific effects to mediate this phenomenon (Gold and Voskuhl, 2016), and pregnancy protection from autoimmunity in animal models depends on intact endocrine regulation of T cells (Engler et al., 2017). However, exogenous

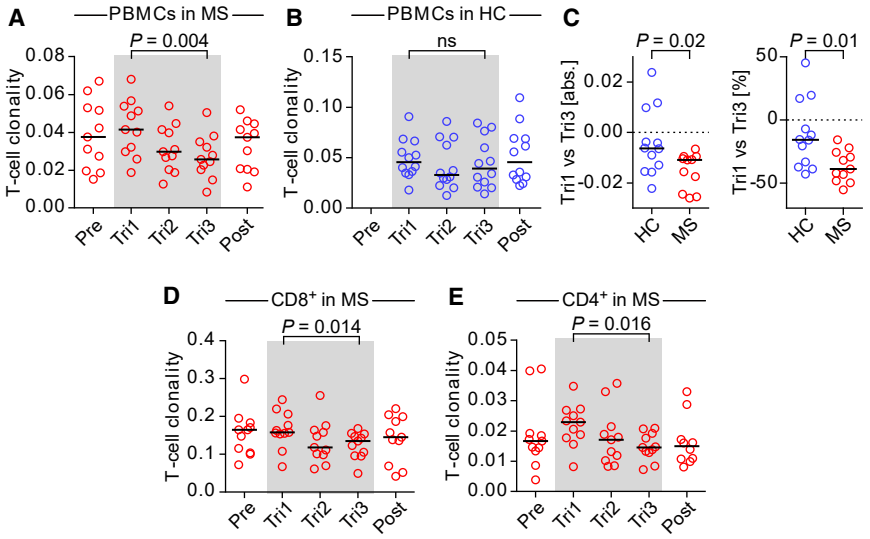

Figure 1. Pregnancy Shifts Clonal Composition of the T Cell Repertoire

(A and B) T cell clonality tracked during pregnancy in PBMCs of female patients with MS (A) and healthy pregnant women (B). A decrease in clonality indicates that the T cell repertoire is less dominated by expanded clones and becomes more evenly distributed.

(C) Degree of absolute and relative change of T cell clonality in PBMCs from trimester 1 to trimester 3, contrasted between MS patients and healthy controls.

(D and E) T cell clonality of $CD8^+$ (D) and $CD4^+$ T cells (E) tracked during pregnancy in MS patients.

Data are shown as individual data points per subject (n = 11, MS; n = 12, HC), including median; p values are derived from a Wilcoxon matched-pairs signed rank test comparing trimester 1 to trimester 3 (A, B, D, and E) and a Mann-Whitney *U* test comparing groups (C).

See also Figures S1 and S2.

application of these hormones does not fully replicate the protective effect of pregnancy on MS (Pozzilli et al., 2015; Voskuhl et al., 2016). Moreover, animal models have indicated that cell-mediated, antigen-specific mechanisms contribute substantially to the establishment of temporary immune tolerance during pregnancy (Engler et al., 2017; Kahn and Baltimore, 2010; Munoz-Suano et al., 2012; Rowe et al., 2012). If evidence were found that pregnancy modulates the human maternal immune system in a specific rather than a global fashion, then this could help to unravel antigen-specific tolerance mechanisms, with potential implications for autoimmune disease, transplantation, and reproductive health.

RESULTS

Pregnancy Alters T Cell Clonality in MS

To test this hypothesis, we employed high-throughput immunosequencing, which allows characterization of the T cell repertoire at the single-clone level, before pregnancy, at three different time points during pregnancy (first, second, and third trimester), and at 3 months postpartum in a cohort of healthy women (n = 12) and a cohort of female MS patients (n = 11). To control for potential confounders, cases and controls were matched for age of the mother, sex of the child, and parity (i.e., number of previous children), with most of the participating women delivering their first child in this study (see Table S1).

As a measure of T cell repertoire diversity, we first examined clonal composition in peripheral blood mononuclear cells (PBMCs) and detected a significant decrease in clonality during pregnancy in MS patients, but not in healthy controls (Figures 1A and 1B). MS patients showed significantly stronger downmodulation of T cell clonality from trimester 1 to trimester 3 compared to controls in terms of both absolute and relative change (Figure 1C), with large effect sizes (d = 1.03 and d = 1.17 for absolute and relative change, respectively). We further confirmed a differential effect of pregnancy on clonality using a general linear model as a sensitivity analysis (main effect for time F = 8.83; degrees of freedom [df] = 2; error df = 20; p = 0.002; effect size partial η^2 = 0.469 as well as a significant time*group interaction F = 3.95; df = 2; error df = 20; p = 0.036; effect size partial η^2 = 0.283). This indicates that during MS pregnancy, the T cell repertoire becomes more evenly distributed and less dominated by expanded clones. Repertoire analysis in $CD4^+$ and $CD8^+$ T cells confirmed decreasing clonality during pregnancy in MS patients in both subsets (Figures 1D and 1E).

Of note, no statistically significant differences in T cell clonality between MS patients and healthy controls (CD8 p = 0.41; CD4 p = 0.08; PBMCs p = 0.48) were observed at the earliest time point (trimester 1), further underscoring the large interindividual variability and the importance of searching for clones in individuals over time rather than cross-sectionally between cases and controls. Moreover, tracking T cell repertoire clonality in n = 3 healthy young non-pregnant women over the course of 13 months showed some fluctuations over time in each individual but no discernable pattern (see Figure S1), lending support to the notion that the pregnancy effect in MS patients is unlikely to be simply explained by random variability. The total number of circulating T cells was unaltered during pregnancy, and few significant changes were observed in relative $CD4^+$ and $CD8^+$ frequencies or their general phenotype (see Figure S2). For example, we observed significant reductions in CD45RO-expressing $CD4^+$ and $CD8^+$ cells of MS patients during pregnancy (but not healthy controls), indicating that predominantly antigen-experienced T cells were downmodulated by pregnancy (Figures S2E and S2F).

Pregnancy Significantly Modulates a Small Number of Private T Cell Clones

Next, we sought to identify which clones were responsible for driving the observed changes in T cell repertoire clonality. Pronounced and statistically significant pregnancy effects were seen for the 25 most abundant clonotypes in each MS patient (Figures 2A and 2C), but not in healthy controls (Figures 2B and 2D). Again, absolute as well as relative downregulation in

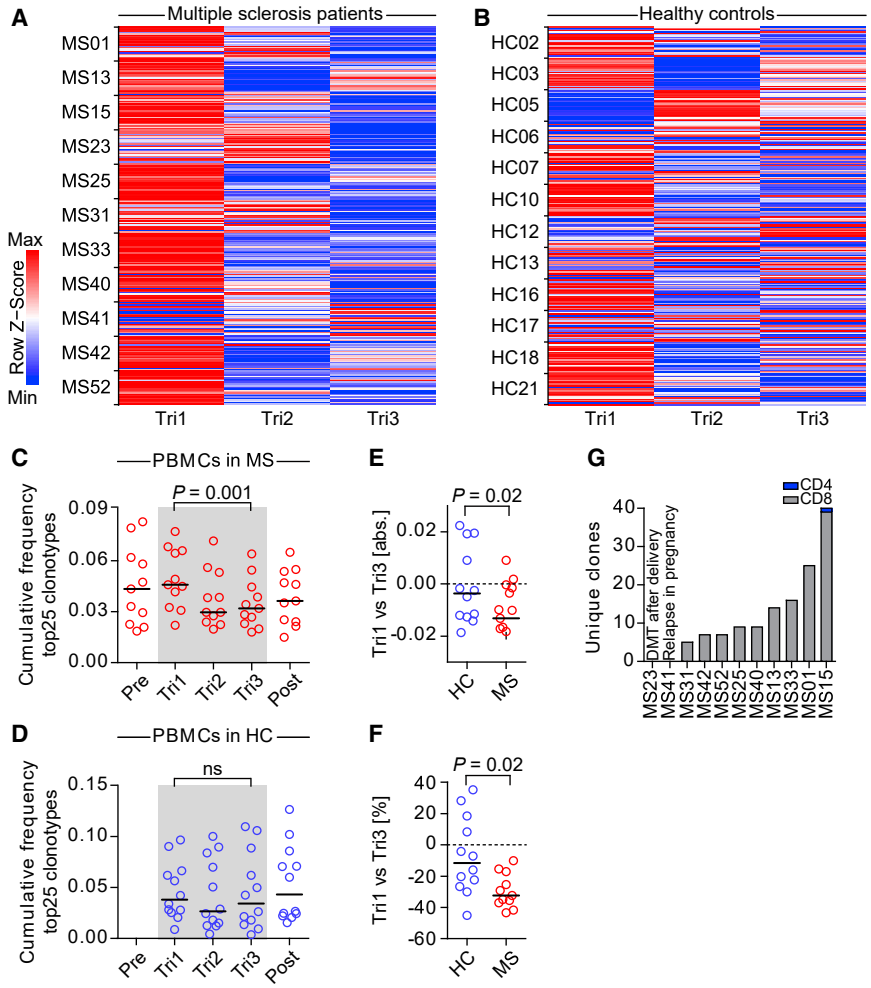

Figure 2. Dynamics of Most Frequent Clonotypes in Pregnancy

(A and B) Heatmap plotting the frequency of the most abundant 25 T cell clonotypes in bulk PBMCs in each MS patient (A) and each healthy control (B) tracked over the course of pregnancy.

(C and D) Quantification of clonotypes shown in (A) and (B), respectively.

(E and F) Degree of absolute (E) and relative change (F) of cumulative frequency of top 25 clonotypes in PBMCs from trimester 1 to trimester 3, contrasted between MS patients and healthy controls.

(G) Number of unique clones identified in each MS patient that significantly contracted from trimester 1 to trimester 3 trimester and re-expanded from trimester 3 to the postpartum time point (adjusted for multiple comparisons).

Data are shown as individual data points per subject (n = 11, MS; n = 12, MS) and median; p values are derived from a Wilcoxon matched-pairs signed rank test comparing trimester 1 to trimester 3 (C and D) and a Mann-Whitney *U* test comparing groups (E and F).

See also Figure S1.

cumulative frequency of the top 25 clonotypes from trimester 1 to trimester 3 was significantly larger in MS patients compared to controls (Figures 2E and 2F).

Then, we identified T cell clones that showed a frequency pattern mirroring the clinical effect of pregnancy in MS, i.e., (1) contracting from trimester 1 to trimester 3, and (2) re-expanding post partum, based on a statistical algorithm and adjusted for multiple comparisons ("candidate clones"; see STAR Methods). We were able to detect clones that followed this pattern in the majority of patients (9 out of 11; Figure 2G), with two notable exceptions: one patient who experienced a relapse during pregnancy (MS41) and another patient (MS23) treated with a disease-modifying therapy (DMT) (dimethyl fumarate) directly after delivery. All other patients had between 5 and 40 unique clones that met our statistical criteria. The vast majority of these clones were found only in a single patient, indicating that they largely represent private clones (see Data S1). By applying the pairSEQ approach (Howie et al., 2015), we were able to successfully identify the corresponding α chain for 1,774 unique T cell clones including most of the candidate clones, thereby yielding the T cell receptor (TCR) sequence described by its V gene, CDR3, and J gene (see Data S1 for a complete list of candidate clones and their frequencies tracked longitudinally over the course of pregnancy and postpartum).

T Cell Receptor Sequencing in an Individual Patient Identifies Relapse-Associated T Cell Clones

Next, we explored the ability of our approach to identify and track relapse-associated clones in an individual patient. Patient MS15 showed the largest number of modulated clones during pregnancy and postpartum (Figure 2G) and also experienced a clinical relapse (optic neuritis) at the 3-month postpartum time point (Figure 3A). This case is particularly informative, as it mirrors the epidemiological pattern of MS disease activity during pregnancy. Therefore, we first identified the clonotypes with the highest frequencies during the postpartum relapse (i.e., top 100 clonotypes) and tracked them retrospectively over the course of pregnancy. These relapse-associated clones showed a statistically significant reduction over the course of pregnancy, with the lowest frequencies in the third trimester (Figures 3B and 3C), observable in both $CD4^+$ and $CD8^+$ cells. Of note, 23 of the 40 candidate clones identified for MS15 above (see Figure 2G) were among the top 100 $CD8^+$ relapse-associated clonotypes. In contrast, there was no significant pregnancy effect on frequencies of myelin- or EBNA-1-specific T cells (Figure S4; Data S2). Moreover, only three EBNA-1-specific (but no myelin-specific) $CD4^+$ T cell clonotypes were detected in the top 100 relapse-associated $CD4^+$ T cell clonotypes (see Figure 3). Together, these data imply that sequencing the TCR during pregnancy in individual patients can yield a substantial number of

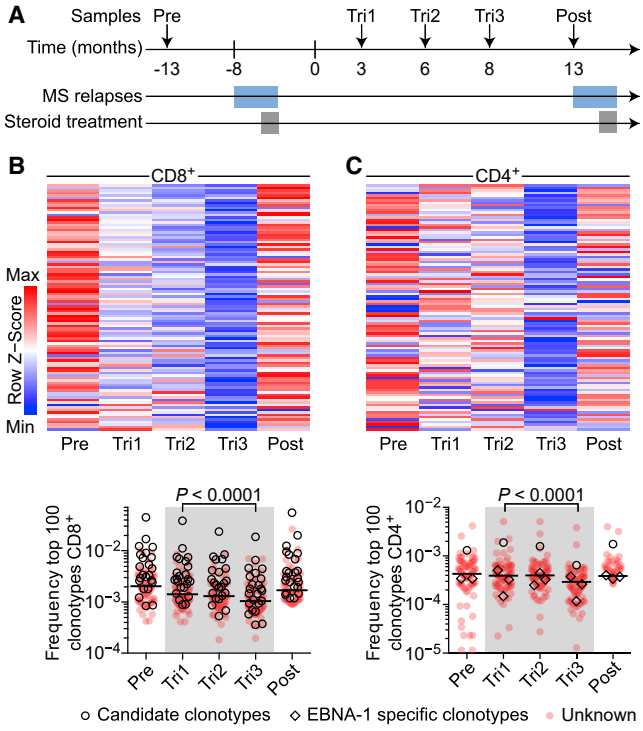

Figure 3. Tracking of Postpartum-Relapse-Associated Clonotypes and Antigen-Specific Clonotypes in Patient MS15

(A) Patient MS15 experienced a postpartum relapse at the time of assessment but had no MS symptoms at any of the time points during pregnancy, therefore providing an opportunity to track relapse-associated and antigen-specific clones during pregnancy.

(B and C) Heatmaps tracking the 100 most abundant T cell clonotypes present during the postpartum relapse over the course of pregnancy are shown for $CD8^+$ (B) and $CD4^+$ compartment (C) with the corresponding quantifications in the panels below. In (B) and (C), candidate clonotypes (see Figure 2G) and EBNA-1-specific clonotypes found among the relapse associated clonotypes are marked as a black circle or black diamond, respectively.

Data are shown as individual clonotypes (n = 100), including median; p values are derived from Wilcoxon matched-pairs signed rank test comparing trimester 1 to trimester 3. HLA genotype of MS15: A *0101/1101; B *0801/3503; DRB1 *0408/1301; DQA1 *0103/03XX; DQB1 *0304/0603.

See also Figures S3 and S4.

potentially relevant clones beyond "classical" putative MS-related antigens such as myelin or EBV.

DISCUSSION

Together, our data demonstrated that pregnancy in MS significantly alters clonal composition of T cells toward a more evenly distributed repertoire driven by selective downregulation of a few largely private T cell clones.

Despite evidence that both $CD4^+$ and $CD8^+$ T cell subsets play a prominent role in MS pathogenesis (Dendrou et al., 2015), identifying disease-relevant and autoantigen-specific T cells in MS has remained challenging for both subsets (Hohlfeld et al., 2016a, 2016b). TCR sequencing approaches have shed light onto the clonal composition of CNS-infiltrating T cells in MS (e.g., Jelcic et al., 2018; Planas et al., 2018), but little is known about the T cell dynamics *in vivo* during times of disease activity and remission. In human subjects in general and pregnant women in particular, such research is obviously limited to correlational analyses, and we cannot infer causality from our data. However, our approach illustrates that pregnancy could be a "window" to the dysregulated immune system leading to the identification of private T cell clones *in vivo* that are associated with disease activity in individual patients. Importantly, our analysis indicates that clones that exhibit the biggest frequency shifts during times of clinical inactivity (e.g., during pregnancy) versus inflammatory exacerbation (e.g., postpartum relapse) are typically private (i.e., unique to individual patients). Moreover, $CD4^+$ T cells specific to putative disease-related antigens in MS such as myelin or Epstein-Barr virus (EBV) do not seem to contribute substantially to the most strongly modulated T cell clonotypes. This highlights the need for personalized approaches to identify and track these clones longitudinally in individual patients rather than cross-sectional case-control comparisons.

We acknowledge some limitations of our study. Patients and healthy controls were well characterized and closely matched on clinical characteristics (but not on human leukocyte antigen [HLA]). However, the sample was still comparatively small, and the study should thus be considered exploratory. Moreover, the functional characteristics and antigen specificity of identified clones are presently unknown. Recent studies have demonstrated pregnancy effects on intracellular signaling (Aghaeepour et al., 2017) and epigenetic signatures (Iannello et al., 2019) in the immune system, and such characterizations in unique T cell clones (Han et al., 2014) could help to better understand the molecular drivers of T cell repertoire changes. Given that the findings we describe herein allow the deciphering of the full receptor based on its V gene, J gene, and CDR3 sequence of the α and β chains of the TCR, such studies are now within reach. As a result, immune profiling during pregnancy could open additional avenues for identifying private T cell clones associated with disease activity in individual patients that could eventually be specifically targeted.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.09.025.

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AUTHOR CONTRIBUTIONS

S.M.G. and M.A.F. conceived the study and acquired funding. C.R., K.P., A.W., S.C.R., A.D., A.H., C.H., P.C.A., M.A.F., and S.M.G. planned and conducted patient recruitment, follow-up, and sample collection. S.M.G., E.T., A.S., C.S., and M.V. designed the experiments. C.R., K.P., S.G., N.S., E.C.Y., M.V., K.C., C.S., R.O.E., and H.S.R. conducted experiments. C.R., E.C.Y., S.G., N.S., J.B.E., K.C., and S.M.G. analyzed the data. C.R., E.C.Y., K.P., S.G., J.B.E., N.S., A.W., M.V., C.S., H.S.R., A.S., K.C., R.O.E., M.A.F., and S.M.G. interpreted the data. C.R., S.G., J.B.E., and S.M.G. designed figures and tables. S.M.G. wrote the manuscript. All authors critically revised the manuscript for intellectual content.

DECLARATION OF INTERESTS

E.C.Y., C.S., H.S.R., K.C., M.V., and R.O.E. have or had employment and equity ownership with Adaptive Biotechnologies. H.S.R. and R.O.E. report patents related to this work. A.S. is a consultant of Miltenyi Biotec, which has patents related to this work. The remaining authors declare no competing interests.

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REFERENCES

Aghaeepour, N., Ganio, E.A., Mcilwain, D., Tsai, A.S., Tingle, M., Van Gassen, S., Gaudilliere, D.K., Baca, Q., McNeil, L., Okada, R., et al. (2017). An immune clock of human pregnancy. Sci. Immunol. *2*, eaan2946.

Bacher, P., Schink, C., Teutschbein, J., Kniemeyer, O., Assenmacher, M., Brakhage, A.A., and Scheffold, A. (2013). Antigen-reactive T cell enrichment for direct, high-resolution analysis of the human naive and memory Th cell repertoire. J. Immunol. *190*, 3967–3976.

Barrett, J.H., Brennan, P., Fiddler, M., and Silman, A.J. (1999). Does rheumatoid arthritis remit during pregnancy and relapse postpartum? Results from a nationwide study in the United Kingdom performed prospectively from late pregnancy. Arthritis Rheum. *42*, 1219–1227.

Carlson, C.S., Emerson, R.O., Sherwood, A.M., Desmarais, C., Chung, M.-W., Parsons, J.M., Steen, M.S., LaMadrid-Herrmannsfeldt, M.A., Williamson, D.W., Livingston, R.J., et al. (2013). Using synthetic templates to design an unbiased multiplex PCR assay. Nat. Commun. *4*, 2680.

Confavreux, C., Hutchinson, M., Hours, M.M., Cortinovis-Tourniaire, P., and Moreau, T.; Pregnancy in Multiple Sclerosis Group (1998). Rate of pregnancy-related relapse in multiple sclerosis. N. Engl. J. Med. *339*, 285–291.

de Man, Y.A., Dolhain, R.J., van de Geijn, F.E., Willemsen, S.P., and Hazes, J.M. (2008). Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study. Arthritis Rheum. *59*, 1241–1248.

Dendrou, C.A., Fugger, L., and Friese, M.A. (2015). Immunopathology of multiple sclerosis. Nat. Rev. Immunol. *15*, 545–558.

DeWitt, W.S., Emerson, R.O., Lindau, P., Vignali, M., Snyder, T.M., Desmarais, C., Sanders, C., Utsugi, H., Warren, E.H., McElrath, J., et al. (2015). Dynamics of the cytotoxic T cell response to a model of acute viral infection. J. Virol. *89*, 4517–4526.

Engler, J.B., Kursawe, N., Solano, M.E., Patas, K., Wehrmann, S., Heckmann, N., Lühder, F., Reichardt, H.M., Arck, P.C., Gold, S.M., and Friese, M.A. (2017). Glucocorticoid receptor in T cells mediates protection from autoimmunity in pregnancy. Proc. Natl. Acad. Sci. USA *114*, E181–E190.

Finkelsztejn, A., Brooks, J.B., Paschoal, F.M., Jr., and Fragoso, Y.D. (2011). What can we really tell women with multiple sclerosis regarding pregnancy? A systematic review and meta-analysis of the literature. BJOG *118*, 790–797.

Gold, S.M., and Voskuhl, R.R. (2016). Pregnancy and multiple sclerosis: from molecular mechanisms to clinical application. Semin. Immunopathol. *38*, 709–718.

Han, A., Glanville, J., Hansmann, L., and Davis, M.M. (2014). Linking T-cell receptor sequence to functional phenotype at the single-cell level. Nat. Biotechnol. *32*, 684–692.

Hohlfeld, R., Dornmair, K., Meinl, E., and Wekerle, H. (2016a). The search for the target antigens of multiple sclerosis, part 1: autoreactive CD4+ T lymphocytes as pathogenic effectors and therapeutic targets. Lancet Neurol. *15*, 198–209.

Hohlfeld, R., Dornmair, K., Meinl, E., and Wekerle, H. (2016b). The search for the target antigens of multiple sclerosis, part 2: CD8+ T cells, B cells, and antibodies in the focus of reverse-translational research. Lancet Neurol. *15*, 317–331.

Howie, B., Sherwood, A.M., Berkebile, A.D., Berka, J., Emerson, R.O., Williamson, D.W., Kirsch, I., Vignali, M., Rieder, M.J., Carlson, C.S., and Robins, H.S. (2015). High-throughput pairing of T cell receptor α and β sequences. Sci. Transl. Med. *7*, 301ra131.

Iannello, A., Rolla, S., Maglione, A., Ferrero, G., Bardina, V., Inaudi, I., De Mercanti, S., Novelli, F., D'Antuono, L., Cardaropoli, S., et al. (2019). Pregnancy epigenetic signature in T helper 17 and T regulatory cells in multiple sclerosis. Front. Immunol. *9*, 3075.

Jelcic, I., Al Nimer, F., Wang, J., Lentsch, V., Planas, R., Jelcic, I., Madjovski, A., Ruhrmann, S., Faigle, W., Frauenknecht, K., et al. (2018). Memory B cells activate brain-homing, autoreactive $CD4^+$ T cells in multiple sclerosis. Cell *175*, 85–100.e23.

Kahn, D.A., and Baltimore, D. (2010). Pregnancy induces a fetal antigen-specific maternal T regulatory cell response that contributes to tolerance. Proc. Natl. Acad. Sci. USA *107*, 9299–9304.

Kirsch, I., Vignali, M., and Robins, H. (2015). T-cell receptor profiling in cancer. Mol. Oncol. *9*, 2063–2070.

Munoz-Suano, A., Kallikourdis, M., Sarris, M., and Betz, A.G. (2012). Regulatory T cells protect from autoimmune arthritis during pregnancy. J. Autoimmun. *38*, J103–J108.

Planas, R., Metz, I., Martin, R., and Sospedra, M. (2018). Detailed characterization of T cell receptor repertoires in multiple sclerosis brain lesions. Front. Immunol. *9*, 509.

Pozzilli, C., De Giglio, L., Barletta, V.T., Marinelli, F., Angelis, F.D., Gallo, V., Pagano, V.A., Marini, S., Piattella, M.C., Tomassini, V., et al. (2015). Oral contraceptives combined with interferon beta in multiple sclerosis. Neurol. Neuroimmunol. Neuroinflamm. *2*, e120.

Robins, H.S., Campregher, P.V., Srivastava, S.K., Wacher, A., Turtle, C.J., Kahsai, O., Riddell, S.R., Warren, E.H., and Carlson, C.S. (2009). Comprehensive assessment of T-cell receptor β-chain diversity in alphabeta T cells. Blood *114*, 4099–4107.

Robins, H., Desmarais, C., Matthis, J., Livingston, R., Andriesen, J., Reijonen, H., Carlson, C., Nepom, G., Yee, C., and Cerosaletti, K. (2012). Ultra-sensitive detection of rare T cell clones. J. Immunol. Methods *375*, 14–19.

Rowe, J.H., Ertelt, J.M., Xin, L., and Way, S.S. (2012). Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. Nature *490*, 102–106.

Samstein, R.M., Josefowicz, S.Z., Arvey, A., Treuting, P.M., and Rudensky, A.Y. (2012). Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. Cell *150*, 29–38.

Sherwood, A., Desmarais, C., and Emerson, R.O. (2015). TCRB time course.https://clients.adaptivebiotech.com/pub/healthy-adult-time-course-TCRB.

Voskuhl, R.R., Wang, H., Wu, T.C., Sicotte, N.L., Nakamura, K., Kurth, F., Itoh, N., Bardens, J., Bernard, J.T., Corboy, J.R., et al. (2016). Estriol combined with glatiramer acetate for women with relapsing-remitting multiple sclerosis: a randomised, placebo-controlled, phase 2 trial. Lancet Neurol. *15*, 35–46.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
CD195 (CCR5) PE (HEK/1/85a)	Biolegend	Cat# 313707; RRID:AB_345307
CD45RO PerCP/Cy5.5 (UCHL1)	Biolegend	Cat# 304221; RRID:AB_1575041
CD28 PE/Cy7 (CD28.2)	Biolegend	Cat# 302926; RRID:AB_10644005
CD197(CCR7) APC (TG8/CCR7)	Biolegend	N/A
CD8 APC/Cy7 (RPA-T8)	Biolegend	Cat# 301016; RRID:AB_314134
CD4 V500 (RPA-T4)	BD	Cat# 560768; RRID:AB_1937323
CD62L Pacific Blue™ (DREG-56)	Biolegend	Cat# 304826; RRID:AB_2186977
CD195(CCR5) FITC (HEK/1/85a)	Biolegend	Cat# 313705; RRID:AB_345305
CD25 PE (BC96)	Biolegend	Cat# 302606, RRID:AB_314276
CD127 PerCP/Cy5.5 (A019D5)	Biolegend	Cat# 351322; RRID:AB_10897104
CD39 PE/Cy7 (A1)	Biolegend	Cat# 328212; RRID:AB_2099950
CCR2 APC (TG5/CCR2)	Biolegend	N/A
CD28 pure - functional grade (15E8)	Miltenyi Biotec	Cat# 130-093-375; RRID:AB_1036134
CD40 pure - functional grade (HB14)	Miltenyi Biotec	Cat# 130-094-133; RRID:AB_10839704
CD3 pure - functional grade (OKT3)	Miltenyi Biotec	Cat# 130-093-387; RRID:AB_1036144
CD4 APC (Vit4)	Miltenyi Biotec	Cat# 130-092-374; RRID:AB_871680
Anti-Biotin FITC (Bio3-18E7)	Miltenyi Biotec	Cat# 130-090-857; RRID:AB_244257
CD69 APC-Vio770 (FN50)	Miltenyi Biotec	Cat# 130-099-907; RRID:AB_2661137
CD14 VioGreen (TÜK4)	Miltenyi Biotec	Cat# 130-096-875; RRID:AB_2660175
CD8 VioGreen (BW135/80)	Miltenyi Biotec	Cat# 130-096-902; RRID:AB_2660905
CD20 VioGreen (LT20)	Miltenyi Biotec	Cat# 130-096-904; RRID:AB_2660933
CD154 VioBlue (5C8)	Miltenyi Biotec	Cat# 130-096-217; RRID:AB_10829767
TNF-α PE (cA2)	Miltenyi Biotec	Cat# 130-091-651, RRID:AB_244203
CD45RA Pacific Blue™ (HI 100)	Biolegend	Cat# 304123; RRID:AB_2174122
CD27 FITC (O323)	Biolegend	Cat# 302806; RRID:AB_31429
Chemicals, Peptides, and Recombinant Proteins		
PepTivator MOG	Miltenyi Biotec	Cat# 130-096-770
PepTivator MBP Isoform 1	Miltenyi Biotec	Cat# 130-096-763
PepTivator PLP	Miltenyi Biotec	Cat# 130-097-274
PepTivator EBV EBNA-1	Miltenyi Biotec	Cat# 130-093-613
RPMI-1640 medium + GlutaMAX	GIBCO, Thermo Fisher Scientific	Cat# 11554516
human AB serum	Sigma Aldrich	Cat# H4522-100ML
TexMACS medium	Miltenyi Biotec	Cat# 130-097-196
IL-2 (Proleukin)	Novartis	N/A
Inside Stain Kit	Miltenyi Biotec	Cat# 130-090-477
Critical Commercial Assays		
Anti-human CD4 particles	BD Imag, BD Bioscience	Cat# 557767; RRID:AB_398629
Anti-human CD8 particles	BD imag, BD Bioscience	Cat# 557766; RRID:AB_398628
DNeasy Blood and Tissue Kit	QIAGEN	Cat# 69506
ImmunoSEQ™ Assay	Adaptive Biotechnologies; Carlson et al., 2013; Robins et al., 2012; Robins et al., 2009	N/A
PairSEQ™ Assay	Adaptive Biotechnologies; Howie et al., 2015	N/A

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
CD154 MicroBead Kit, human	Miltenyi Biotec	Cat# 130-092-658
CD3 MicroBeads, human	Miltenyi Biotec	Cat# 130-050-101
MS columns	Miltenyi Biotec	Cat# 130-042-201
Deposited Data		
Longitudinal MS and HC pregnancy TCR seq data	This paper	https://doi.org/10.21417/B7B012
Time course healthy female TCR seq data	Sherwood et al., 2015	https://doi.org/10.21417/B7J01X
Software and Algorithms		
R version 3.2	R Project for Statistical Computing	RRID:SCR_001905; http://www.r-project.org/
FlowJo	FlowJo	RRID:SCR_008520; https://www.flowjo.com
GraphPad Prism 5	GraphPad Software	RRID:SCR_002798; https://www.graphpad.com/
Uniform Manifold Approximation and Projection (UMAP) Plug-in to FlowJo	FlowJo	arXiv:1802.03426; https://www.flowjo.com/exchange/
ImmunoSEQ™ Analyzer 2.0	Adaptive Biotechnologies	N/A; https://www.adaptivebiotech.com/products-services/immunoseq/immunoseq-analyzer/
ImmunoSEQ™ Analyzer 3.0	Adaptive Biotechnologies	N/A; https://www.adaptivebiotech.com/products-services/immunoseq/immunoseq-analyzer/

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Stefan M. Gold (stefan.gold@zmnh.uni-hamburg.de). This study did not generate new unique reagents or materials.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Subjects and recruitment

MS Patients were recruited through the Multiple Sklerose Tagesklinik of the Universitätsklinikum Hamburg-Eppendorf. Healthy controls were recruited through the Klinik für Geburtshilfe und Pränatalmedizin, Universitätsklinik Hamburg-Eppendorf. The study was approved by the local ethics commission (Ethik-Kommission der Hamburger Ärztekammer; Approval No: PV3558, No: PV3694). All participants gave informed consent and were remunerated for time and effort. Female MS patients were selected from our study cohort based on sample availability and confirmed MS diagnosis. Healthy women were selected to match age, sex of the child, and parity (see Table S1). All mothers had single pregnancies. All MS patients were free of disease-modifying therapies (DMTs) during pregnancy. At the pre-pregnancy time point, one patient received DMT (interferon β-1a; MS42) and one patient was in relapse management and received steroid treatment (1mg for 3 days; blood sampled before second infusion; MS23). All but two patients (MS 41 DMT (interferon β-1a), MS23 DMT (dimethyl fumarate)) remained free of DMTs until after the last blood sample (three months post partum). Details on DMTs are provided in Table S1.

METHOD DETAILS

Blood sampling and processing

Blood samples of MS patients were collected before, during and after pregnancy: before conception (Pre), in trimester 1 between week 10 and 14 of gestation (Tri1), in trimester 2 between week 22 and 24 (Tri2), in trimester 3 between week 30 and 36 (Tri3) and three months post partum (Post). Healthy controls gave blood samples at the same time points during pregnancy and post partum (but pre-pregnancy samples were not available for controls). Blood was collected in EDTA coated tubes (Sarstedt, Nümbrecht, Germany) and peripheral blood mononuclear cells (PBMCs) were isolated by a 30min gradient centrifugation at 863xg using Biocoll Separating Solution (Biochrom, Berlin, Germany), followed by two washing steps. Cells were then cryopreserved in RPMI medium (PAN-Biotech, Aidenbach, Germany) with 25% fetal calf serum (Biochrom) and 10% DMSO (AppliChem, Darmstadt, Germany)

and stored in liquid nitrogen until further analysis. During experimental procedures, the experimenters were not blinded. A differential blood count was performed on full blood using a Coulter AC*T Diff (Beckmann Coulter, Brea, CA, USA).

Flow cytometry

After a short resting period, fresh blood samples were incubated with antibody cocktail (CD45RA PB (HI 100), CD27 FITC (O323), CCR5 PE (HEK/1/85a), CD45RO PerCP/Cy5.5 (UCHL1), CD28 PE/Cy7 (CD28.2), CCR7 APC (TG8/CCR7), CD8 APC/Cy7 (RPA-T8), (all Biolegend, San Diego, CA, USA); CD4 V500 (RPA-T4; BD Bioscience, San José, CA, USA)) or (CD62L PB (DREG-56), CCR5 FITC (HEK/1/85a), CD25 PE (BC96), CD127 PerCP/Cy5.5 (A019D5), CD39 PE/Cy7 (A1), CCR2 APC (TG5/CCR2), (all Biolegend); CD4 V500 (RPA-T4; BD Bioscience)) in the dark at room temperature, then erythrocytes were lysed with BD FACS Lysing Solution (BD Bioscience) and washed twice in PBS (520xg, 5min, 4°C). Samples were measured on a FACS Canto II (BD Bioscience). Data were analyzed using FloJo software (FlowJo, LLC., Ashland, OR, USA). Dimensionality reduction was performed on a concatenated dataset after downsampling lymphocytes positive for either CD8, CD4, or both to 10000 events (approx. 750000 events). Uniform Manifold Approximation and Projection (UMAP) plugin from FlowJo was used (Euclidian distance, nearest neighbor: 15 and minimum distance: 0.5, channels: CD4, CD8, CCR5, CD45RO, CD45RA and CCR7). To visually display change over time, another downsampling to 20000 events of the concatenated dataset was performed. Classical manual gating was performed for depicted T cell subsets.

Identification of antigen-specific CD4$^+$ T cells

Fresh PBMCs from patient MS15 were isolated by density gradient centrifugation (Biocoll, Biochrom) and resuspended at a concentration of 2x10^7/ml in RPMI 1640 medium supplemented with GlutaMAX (GIBCO, Thermo Fischer Scientific Darmstadt, Germany) and 5% (v/v) human AB serum (Sigma Aldrich, Schnelldorf, Germany). Myelin and EBNA-1-specific CD4$^+$ T cells were enriched according to the Antigen-Reactive T Cell Enrichment (ARTE) protocol (Bacher et al., 2013) with modifications as described. 9x10^7 cells were stimulated for 6 h with MOG, MBP1 and PLP peptide pools and 3.8x10^7 cells were stimulated with EBNA-1 peptide pool (each 0.6 nmol/peptide/ml) in the presence of 1 μg/ml CD40 (clone HB14) functional grade pure antibody (all Miltenyi Biotec, Bergisch Gladbach, Germany). Activated CD154$^+$ T cells were magnetically enriched (CD154 MicroBead Kit and MS columns, Miltenyi Biotec) and stained with CD4 APC (Vit4), CD154 (via anti-Biotin-FITC, Bio3-18E7), CD69 APC-Vio770 (FN50), CD14 VioGreen (TÜK4), CD8 VioGreen (BW135/80) and CD20 VioGreen (LT20) antibodies (all Miltenyi Biotec). To further increase purity, antigen-reactive CD4$^+$/CD154$^+$/CD69$^+$ T cells were FACS-sorted (Influx cell sorter; BD Biosciences). About 2500 (myelin) or 1000 (EBNA-1) isolated cells were expanded for 8 days with 2.5x10^6 irradiated allogenic feeder cells in a 48-well plate in TexMACS medium (Miltenyi Biotec) supplemented with 30 ng/ml anti-CD3 (OKT-3; Miltenyi Biotec), 200 IU/ml IL-2 (Proleukin; Novartis, Nürnberg, Germany), 100 IU/ml Penicillin, 100 μg/ml Streptomycin (both Sigma Aldrich), 20 μM β-Mercaptoethanol (GIBCO, Themo Fischer Scientific) and 5% human AB serum. The cells were split and medium was replenished as needed. After resting in RPMI 1640 medium + 5% human AB serum for 2 days, expanded cells were restimulated for 7 hours with the initial myelin antigens or EBNA-1 in the presence of 1 μg/ml CD28 functional grade pure antibody (15E8; Miltenyi Biotec) and autologous CD3-depleted PBMCs in a 1:1 ratio (CD3 MicroBeads, Miltenyi Biotec). After 3 hours, 1 μg/ml Brefeldin A (Sigma Aldrich) was added. The cells were stained with CD4 APC and CD14 VioGreen, fixated and permeabilized (Inside Stain Kit, Miltenyi Biotec) and stained intracellularly with CD154 VioBlue (5C8) and TNF-α PE (cA2, both Miltenyi Biotec). Activated CD154$^+$/TNF-α$^+$ T cells and non-activated CD154$^-$/TNF-α$^-$ T cells were FACS-sorted. Cells at several steps throughout the expansion procedure were shock-frozen in liquid nitrogen (1.5x10^5 cells at time points 5/6 and 5x10^5 cells at time points 1-4, see Figure S3) for later DNA isolation and TCR sequencing as described below.

Cell purification and DNA extraction

PBMCs were thawed, counted and separated into 3 parts: at least 10^6 PBMCs were directly pelleted and shock frozen, the other 2 parts were used for magnetic cell sorting of CD4$^+$ and CD8$^+$ cells. CD4$^+$ and CD8$^+$ cells were isolated separately in parallel procedures using positive selection kits (BD Imag; BD Bioscience) according to the manufacturer's instructions (using 30μL of magnetic particles for up to 10^7 cells). After isolation, CD4$^+$ and CD8$^+$ cells were pelleted and shock-frozen. To extract DNA, cell pellets were thawed and processed with the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol for tissue, then stored at −20°C until shipment.

Immunosequencing

Immunosequencing of the CDR3 regions of human TCRβ chains was performed using the ImmunoSEQ^{TM} Assay (Adaptive Biotechnologies, Seattle, WA, USA). Extracted genomic DNA was amplified in a bias-controlled multiplex PCR, followed by high-throughput sequencing. Sequences were collapsed and filtered in order to identify and quantitate the absolute abundance of each unique TCRβ CDR3 region for further analysis as previously described (Carlson et al., 2013; Robins et al., 2009, 2012).

TCRα × TCRβ pairs were identified using the PairSEQ^{TM} Assay. Here, the TCRα and TCRβ loci are immunosequenced on a single 96 well plate, using the ImmunoSEQ^{TM} Assay as described above. This yields an occupancy vector with 96 components for each TCRα and TCRβ rearrangement. Comparison with the null probabilities given by random subset sampling allow statistically confident pairing of TCRα and TCRβ with sufficiently similar occupancy vectors, and a multiple testing correction procedure involving a simulated null distribution enforces a false discovery rate of 1% (Howie et al., 2015).

QUANTIFICATION AND STATISTICAL ANALYSIS

Clonality was defined as 1- Pielou's evenness (Kirsch et al., 2015) and was calculated on productive rearrangements by: $1 + (\sum_{i}^{N} p_i \log_2(p_i) / \log_2(N))$ where p_i is the proportional abundance of rearrangement i and N is the total number of rearrangements. Clonality values range from 0 to 1 and describe the shape of the frequency distribution: clonality values approaching 0 indicate a very even distribution of frequencies, whereas values approaching 1 indicate an increasingly asymmetric distribution in which a few clones are present at high frequencies. Changes in TCR clonality, clonotype frequencies, lymphocyte frequencies and phenotype during pregnancy were tested using Wiloxon matched-paris signed rank test (trimester 1 versus trimester 3). Group differences of absolute and relative change of clonality and the cumulative frequency of top clonotypes was performed using Mann-Whitney-U test. In the afore mentioned tests, P-values < 0.05 were considered statistically significant. Statistical details of each experiment are reported in the respective figure legends. Statistical analysis was performed in R version 3.2. and GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). So called "candidate clones" were defined as clones that had significantly lower frequency in trimester 3 compared to trimester 1 and significantly higher frequency post partum compared to trimester 3, i.e. these clones contracted from trimester 1 to trimester 3 and expanded from trimester 3 to post partum. These clones were identified based on a statistical method performed as described (DeWitt et al., 2015), with minor variations to statistical parameters involving implantation of a minimum total template count filter of 5 templates, and incorporation of multiple testing correction with the Benjamini-Hochberg (BH) method. After multiple-testing correction, the threshold of significance was $\alpha < 0.01$ on the BH adjusted P-values.

Clonotype frequency data was exported with ImmunoSEQ Analyzer 3.0 (Adaptive Biotechnologies) as frequency of all unique productive TCRβ CDR3 amino acid sequences. Candidate clonotypes frequencies (see Data S1 and S2) were extracted with ImmunoSEQ Analyzer 2.0 (Adaptive Biotechnologies), displaying frequency of clones as percentage of all rearrangements.

DATA AND CODE AVAILABILITY

The complete data from this study are available for download and analysis at an open-access, shareable repository (ImmuneACCESS). Data may be accessed using the following link after creating a free account: https://doi.org/10.21417/B7B012.

Data on time course of healthy females is accessible at: https://doi.org/10.21417/B7J01X (Sherwood et al., 2015). No new code was generated in this study.