Enhanced gut homing receptor expression of unswitched memory B cells in rheumatoid arthritis

Sirs,

Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory disorder characterised by the presence of autoantibodies such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibody (ACPA). The importance of the gut microbiome in autoimmunity related to RA has been implicated in both mice models and human disease. In the K/BxN mouse model, a single segmented filamentous bacteria induces autoantibodies and autoimmune arthritis (1). Also, gut dysbiosis and its correlation to autoantibodies have been reported in RA patients (2). These observations suggest the possibility that the intestinal interaction between the gut microbiome and RA B cells could be related to autoantibody production. In systemic lupus erythematosus (SLE) B cells, decreased expression of the gut homing receptor integrin α4β7 results in impaired depletion of autoreactive immature B cells in gut-associated lymphoid tissues (GALT) (3). However, there is little information about the gut access of RA mature B cell subsets.

We analysed the expression of the gut homing receptor integrin α4β7 on peripheral blood B cell subsets with flow cytometry. Lymphocytes use integrin α4β7 to extravasate from blood to GALT by interacting with the ligand MAdCAM-1 (4). RA patients (n=16) and healthy donors (HD, n=16) gave written informed consent prior to participation in our immunophenotyping analysis (5). In addition, five SLE patients who provided written informed consent were included as a disease control for RA. This study was approved by the Ethical Committee of the University of Tokyo Hospital (no. 10154). Briefly, peripheral blood mononuclear cells were isolated and analysed with flow cytometry. B cells were classified according to the standardised immunophenotyping protocol (5, 6). The expression of integrin β7 represents the expression of integrin α4β7(7). APC rat anti-human integrin β7 (FIB504, BD) was used for integrin β7 detection. Consistent with a previous report that integrin α4β7 expression on RA mature B cells is comparable to healthy controls (3), the expression of integrin β7 in RA was not altered in most subsets. However, we observed an enhanced integrin β7 (ib7) expression specifically in unswitched $CD3^-CD19^+IgD^+CD27^+$ memory B cells (unSwMB) in RA (Fig. 1A). This increase was not observed in SLE patients. Interestingly, increased integrin β7 on unswitched memory B cells in RA was significantly correlated with RF titers, not with ACPA titers (Fig. 1B). Also, of the analysed B cell subsets, only unswitched memory B cells' integrin β7 expression showed a significant correlation with RF titer. These results suggest a relationship between the enhanced gut homing receptor expression on unswitched memory B cells and RF production in RA.

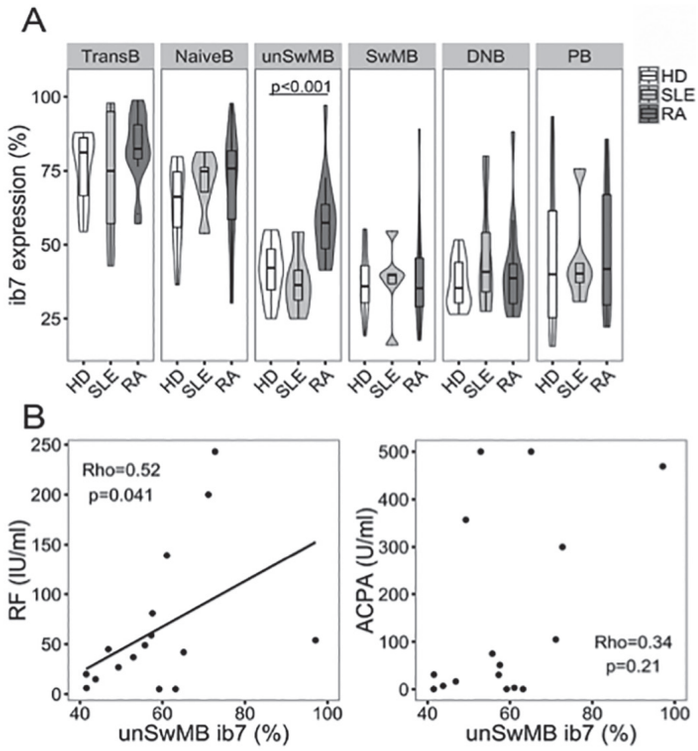

Fig. 1. Integrin β7 expression in RA B cell subsets.
A. The expression ratio of integrin β7 on B cell subsets was compared between HD (n=16), SLE (n=5), and RA (n=16). Comparison with HD was performed with the Mann-Whitney U-test. Only unswitched memory B cells in RA showed a significant difference (p=0.00019). Subset definitions are as follows: transitional B cells (TransB) $CD3^-CD19^+CD24^{high}CD38^{high}$, naive B cells (NaiveB) $CD3^-CD19^+IgD^+CD27^-$, unswitched memory B cells (unSwMB) $CD3^-CD19^+IgD^+CD27^+$, switched memory B cells (SwMB) $CD3^-CD19^+IgD^-CD27^+$, double-negative B cells (DNB) $CD3^-CD19^+IgD^-CD27^-$, plasmablasts (PB) $CD3^-CD19^+IgD^-CD27^{high}CD38^{high}$.
B. In RA, the expression ratio of integrin β7 on unswitched memory B cells (unSwMB ib7) was positively correlated with rheumatoid factor (RF) titer, but not with anti-cyclic citrullinated peptide antibody (ACPA) titer (n=16). The correlation was evaluated by Spearman's rank correlation coefficients.

Human $IgM^+IgD^+CD27^+$ unswitched memory B cells have been reported to be natural memory cells and circulating marginal zone B cells, which are rapidly activated to secrete immunoglobulin in response to T cell-independent antigens (8). RF is an autoantibody that binds to Fc fragments of IgG or denatured IgG. Most of the RFs are of the IgM isotype and could be the result of B cell activation and T cell-independent immunoglobulin production (9). Thus, unswitched memory B cells could be a major source of RF, although *in vitro* studies of sorted populations are required to prove this hypothesis. Transient induction of RF has been reported in bacterial infections (9), and combined elevation of IgM RF and IgA RF is highly specific to RA(10). Altered gut microbiota in RA (2) and increased gut homing receptor of unswitched memory B cells could contribute to RF production. These observations suggest that RF is derived from mucosal immunity in RA, and the induction mechanism of RF may be different between RA and SLE. Further studies are needed to analyse integrin β7 expression on B cells in other autoimmune diseases with RF production, such as Sjögren's syndrome. Although our analysis was limited to integrin β7, more comprehensive analysis of other integrins, such as integrin α4β1, will help elucidate the mechanism of cell adhesion and migration of B cells in RA.

In conclusion, we observed an increased expression of the gut homing receptor integrin α4β7 only in unswitched memory B cells in RA. In addition, this expression was correlated with RF titer. Although our observation is based on a limited number of participants, our data suggest the possibility that enhanced gut homing receptor expression on unswitched memory B cells in RA plays a role in RF production.

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