Research article

B lymphocytopenia in rheumatoid arthritis is associated with the DRB1 shared epitope and increased acute phase response

Ulf Wagner, Sylke Kaltenhäuser, Matthias Pierer, Bernd Wilke, Sybille Arnold and Holm Häntzschel

Department of Medicine IV, University of Leipzig, Leipzig, Germany

Corresponding author: Holm Häntzschel (e-mail: haentzho@medizin.uni-leipzig.de)

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Abstract

The influence of HLA DRB1 alleles on B-cell homeostasis was analyzed in 164 patients with rheumatoid arthritis (RA). The percentages of $CD19^+$ B lymphocytes determined in the peripheral circulation of 94 retrospectively recruited RA patients followed a bimodal distribution. Two frequency peaks ($B\text{-}cell_{low}$ patients and $B\text{-}cell_{high}$ patients) were separated by the population median of a B-cell frequency of 8.5% of all lymphocytes. Human leucocyte antigen genotyping revealed that the $B\text{-}cell_{low}$ patients were more frequently positive for the RA-associated HLA DRB1 shared epitope (SE) than were $B\text{-}cell_{high}$ patients. Accordingly, SE-positive patients had lower CD19 percentages in the rank-sum analysis when compared with SE-negative patients, and were markedly B lymphocytopenic when compared with a healthy control group. To confirm the differential frequencies of $CD19^+$ B cells, absolute numbers in peripheral blood were determined prospectively in a cohort of 70 RA patients with recent onset disease. SE-positive patients were found to have lower absolute numbers of circulating $CD19^+$ B cells. B-cell counts below the mean of the study population were associated with higher acute phase response and with increased levels of rheumatoid factor IgA. No correlation between absolute numbers of circulating B cells and radiographic progression of joint destruction was seen. The influence of immunogenetic parameters on B-cell homeostasis in RA reported here has not been described previously. The clinical relevance of B lymphocytopenia in SE-positive RA will be further investigated in longitudinal studies.

Keywords: antibodies, B lymphocytes, major histocompatibility complex, rheumatoid arthritis

Introduction

The production of rheumatoid factor (RF) IgM is one of the hallmarks of RA and is frequently associated with more severe disease. Other autoantibodies detectable either in serum or in synovial fluid of RA patients include antinuclear factors [1,2], antineutrophil cytoplasmic antibodies [1–5], antibodies against native collagen type II [6], citrullinated peptides [7] and gp130-RAPS [8], and others.

The relevance of autoantibody-producing, autoreactive B cells for the pathogenesis of RA has recently been highlighted by the success of therapeutic B-cell depletion [9]. Although the precise consequences of the production of RF and other autoantibodies are not known to date, there is evidence for immune-complex-mediated damage to endothelial cells in rheumatoid vasculitis [10] as well as evidence for a role for complement activation via the classical pathway in the tissue damage observed in RA [11]. More recently, animal models have provided further evidence for the pathogenetic relevance of autoantibody production [12] and of the formation of immune complexes and their subsequent binding to Fc receptors in rodent erosive polyarthritis models resembling RA [13].

RF production in RA is thought to occur in the synovial infiltrate in affected joints, which contains follicular structures resembling the germinal centers of secondary lymphoid organs, although those structures can be found in

$B\ cell_{high}$ = patients with high CD19 percentages, above 8.5% of circulating lynphocytes; $B\ cell_{low}$ = patients with low CD19 percentages, below 8.5% of circulating lymphocytes; $CD19_{high}$ = patients with absolute B cell counts above the mean of the study population (110 cells/ml); $CD19_{low}$ = patients with absolute B cell counts below the mean of the study population (110 cells/ml); CRP = C-reactive protein; DMARD = disease modifying antirheumatic drug; ELISA = enzyme-linked immunosorbent assay; MHC = major histocompatibility complex; PCR = polymerase chain reaction; RA = rheumatoid arthritis; RF = rheumatoid factor; SE = HLA DRB1 shared epitope.

only 25% of patients [14]. This view has been supported by evidence for affinity maturation of B-cell clones isolated directly from such structures [15] or from synovial tissue [16,17]. Alternatively, RF production has also been reported for B cells isolated from the peripheral circulation of RA patients [18,19], and activated B cells from synovitic joints have been found to be able to leave the germinal center-like structures and recirculate into the peripheral circulation [20,21].

In the present study, the accessible B lymphocytes in the peripheral circulation were analyzed by flow cytometry to determine global parameters of the peripheral B-cell homeostasis in RA patients. Aggravated B-cell autoreactivity has been suggested to preferentially occur in patients positive for RA-associated DRB1*04 alleles, which were found to be associated not only with production of RF [22], but also production of a variety of other autoantibodies [2,6,23,24]. The goal of the present study was therefore the analysis of frequencies and distributions of B-lymphocyte subpopulations, and the comparison of patients positive and negative for RA-associated HLA DRB1 alleles.

Patients and methods

Ninety-four patients with long-standing RA according to the 1987 American College of Rheumatology diagnostic criteria [25] were recruited into a cross-sectional, retrospective study. Clinical data collected included parameters of disease activity (swollen and tender joint count, duration of morning stiffness), radiological findings from hand and foot radiographs taken at study enrollment, past and present medications received, and presence of extra-articular symptoms (detailed descriptions are presented in Table 1). As a control group, 30 healthy individuals aged between 20 and 73 years (mean age, 52.1 years; 21 women and nine men) were asked to participate in the study.

For the prospective analysis of absolute lymphocyte numbers, 70 RA patients who had been followed since the onset of their disease and who have been described previously were recruited [26]. Detailed clinical and laboratory data, and serial radiographs of hands and feet were available for all patients (see Table 1).

Serum and whole blood samples were obtained from each patient. Laboratory parameters determined in both study populations included the serum concentration of class-specific RF IgM and RF IgA, the presence and titer of antinuclear factor, antibodies against double-stranded DNA, serum immunoglobulin concentrations for the IgM, IgG and IgA isotypes, and concentrations of circulating immune complexes. For details on standard laboratory tests and the flow cytometric analysis performed, see Supplementary material.

The determination of absolute lymphocyte numbers ($CD19^+$ B cells and $CD4^+$ T cells) was performed using true count technology (TRUCOUNT®; Becton Dickinson, Heidelberg, Germany) according to the manufacturer's instructions. Absolute numbers of cells were calculated by dividing the number of positive cellular events by the number of bead events and subsequently multiplying by the TRUCOUNT® bead concentration.

HLA BRB1 genotyping and statistical analysis was performed as described previously [27] (see Supplementary material).

Results

The frequency of $CD19^+$ B cells is dependent on HLA DRB1

In the initial, retrospective study, the frequency of B cells was determined as a percentage of $CD19^+$ lymphocytes from total T lymphocytes and B lymphocytes combined ($CD3^+$ + $CD19^+$ lymphocytes). The CD19 percentages found in RA patients showed a bimodal distribution, with two separate subpopulations passing the Kolmogorov–Smirnov normality test for a Gaussian distribution (Kolmogorov–Smirnov distance = 0.092 [$P > 0.2$] for the population below 8.5% $CD19^+$ cells; Kolmogorov–Smirnov distance = 0.148 [$P > 0.05$] for the population above 8.5% $CD19^+$ cells) (shown in Fig. 1a).

When this cut-off value of 8.5% $CD19^+$ cells was used to separate patients into those with low CD19 percentages (B $cell_{low}$) and those with high CD19 percentages (B $cell_{high}$), a differential human leucocyte antigen association with this phenomenon became apparent. Of the 58 patients in the B-$cell_{low}$ group 58.6% were positive for a RA-associated DR4 allele (SE $DR4^+$), compared with only 33.3% of the 36 patients in the B-$cell_{high}$ group ($P = 0.03$). This difference was even more pronounced when the two groups were analyzed for the presence of the shared epitope (SE-positive), which combines the RA-associated DRB1 alleles DR4 and DR1. Of the B-$cell_{low}$ patients 84.5% were SE-positive, in contrast to only 50% of the B-$cell_{high}$ patients ($P < 0.001$).

Determination of the percentage of $CD19^+$ B cells from total lymphocytes in the healthy control group revealed that SE-positive RA patients had decreased percentages of B cells in the peripheral circulation when compared with healthy individuals (mean, 7.6% versus 10.8%, $P = 0.02$) (see Fig. 1b). In contrast, SE-negative RA patients had higher B-lymphocyte percentages than the controls (mean, 15.8% versus 10.8%, $P = 0.05$).

In the RA patients, no difference was seen between B-$cell_{low}$ patients and B-$cell_{high}$ patients in the clinical parameters analyzed (see Supplementary material) or in the usage of disease modifying antirheumatic drugs (DMARDs) or prednisolone at either the time of analysis or in the past.

Table 1

Characteristics of the two patient cohorts

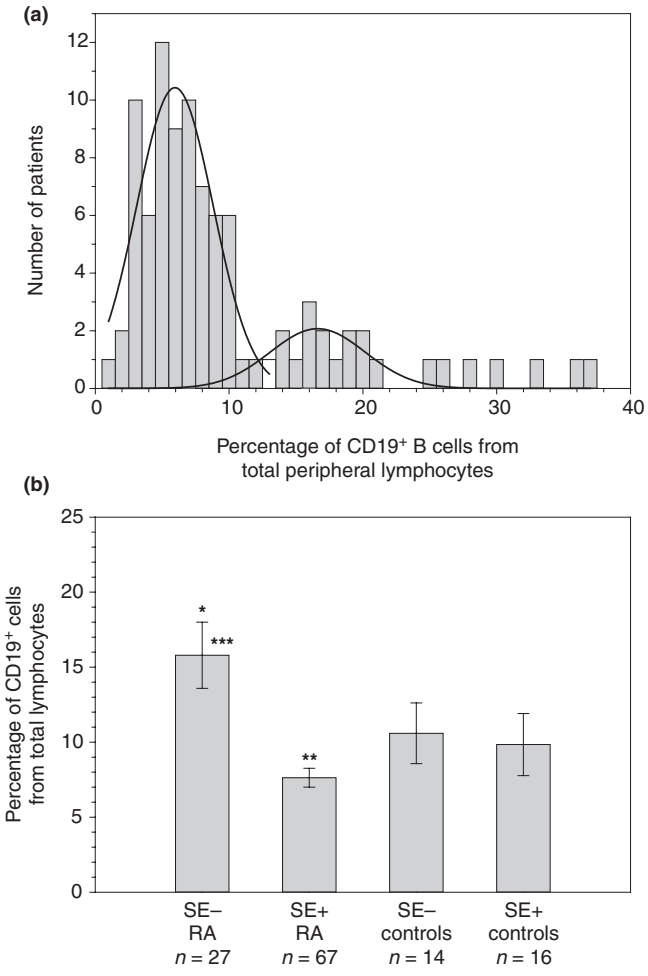
	Retrospective study	Prospective cohort
Number of patients (female/male)	94 (73/14)	70 (53/17)
Age at disease onset (years) [mean (range)]	45.8 (20–77)	51.9 (19–74)
Disease duration (years) [mean (range)]	16.7 (1.4–61)	4.44 (4.1–6.7)
ESR (mm/h) [mean (range)]	34.2 (2–100)	23.8 (3–76)
C-reactive protein (mg/l) [mean (range)]	34.3 (0–190)	13.1 (0–116.5)
Patients positive for RF IgM [*n* (%)]	51 (54.3)	42 (60)
RF IgM concentration (IU/ml) [mean (range)]	344.6 (0–3680)	245.2 (0–3430)
Patients positive for RF IgA [*n* (%)]	52 (55.3)	3 (48.6)
RF IgA concentration (IU/ml) [mean (range)]	105.6 (0–600)	71.8 (0–600)
Patients positive for ANF [*n* (%)]	56 (59.5)	55 (78.6)
Extra-articular manifestations		
Rheumatoid nodules [*n* (%)]	28 (29.8)	5 (7.1)
Keratokonjunctivitis sicca [*n* (%)]	30 (32)	10 (14.3)
Polyserositis [*n* (%)]	2 (2.1)	0
Interstitial pulmonary fibrosis [*n* (%)]	1 (1.1)	0
Immunogenetics		
DRB1*01$^+$ [*n* (%)]	21 (22)	19 (27.1)
SE$^+$ DR4$^+$ [*n* (%)]	46 (49)	32 (45.7)
SE$^+$ DR4$^+$ homozygotes [*n* (%)]	15 (16.0)	8 (11.4)
SE$^+$ [*n* (%)]	67 (71)	51 (72.8)
SE compound homozygotes [*n* (%)]	24 (25.5)	12 (17.1)
Therapy		
Methotrexate	57	56
Cyclosporine A	9	6
Azathioprine	6	0
Chlorochine/sulfasalazine/gold salts intramuscularly	6	5
Cyclophosphamide	7	0
No DMARD	9	7
Number of DMARD used [mean (range)]	2 (0–5)	1 (0–3)

ANF, antinuclear factors; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; SE$^+$, presence of the shared epitope on a DRB1*01 or DRB1*04 allele; SE$^+$ DR4$^+$, presence of the shared epitope on a DRB1*04 allele; SE compound homozygotes, presence of SE on both chromosomes. Clinical characterization at the time of flow cytometric analysis, immunogenetic markers and disease-modifying antirheumatic drugs (DMARDs) received in the two study populations.

Absolute B-cell counts prospectively analyzed in RA patients

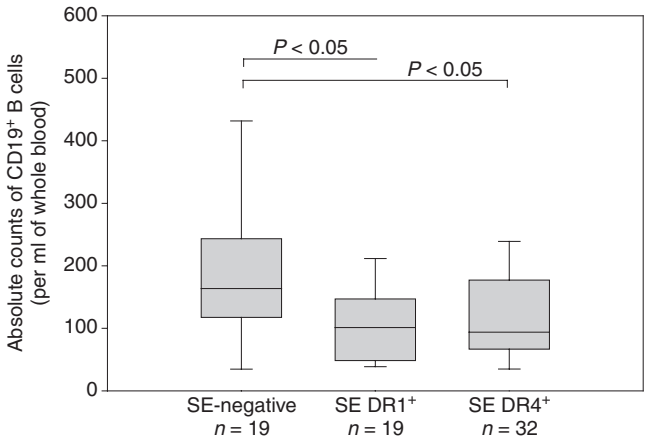
In the prospective study of RA patients with recent-onset disease, TRUCOUNT® technology in a whole blood assay was applied to determine absolute numbers of both B lymphocytes and T lymphocytes. At the time of analysis, patients had a mean disease duration of 4.4 years (Table 1). HLA DRB1 genotyping of the patients confirmed that SE-positive patients have lower absolute numbers of CD19$^+$ B cells in the peripheral circulation when compared with SE-negative patients (median cell number per milliliter of whole blood, 94.4 versus 163.7; interquartile range, 56.4–159.7 versus 117.4–243.4 [$P = 0.022$]). Accordingly, patients with B-cell counts below the mean of the

Figure 1



(a) Histogram depicting the distribution of B-cell frequencies in the peripheral circulation from 94 rheumatoid arthritis (RA) patients. The percentage of $CD19^+$ cells from total peripheral lymphocytes is plotted on the *x* axis, and the number of patients in each frequency range is plotted on the *y* axis. The overlays represent the Gaussian frequency distributions fitted to the two populations. **(b)** Percentage of $CD19^+$ B cells in the peripheral circulation in patients negative (SE–) and positive (SE+) for the RA-associated shared epitope and in age-matched healthy controls. Bars depicts mean and standard error of the mean. * $P = 0.05$ compared with healthy controls, ** $P = 0.02$ compared with healthy controls, *** $P < 0.001$ compared with SE-positive RA patients.

Figure 2



B-cell counts in the peripheral circulation of 70 prospectively followed rheumatoid arthritis (RA) patients determined after a mean disease duration of 4.4 years. Absolute numbers of $CD19^+$ B cells are depicted to exclude shifts in the B-cell/T-cell ratio of patients expressing the RA-associated shared epitope on a DR4 allele (SE $DR4^+$), of patients expressing DR1 but not a RA-associated DR4 allele (SE $DR1^+$), and of patients negative for the SE (SE-negative). Box plots depict the median and interquartile range.

study population (110 cells/ml, $CD19_{low}$) were more frequently positive for the shared epitope (88.2% versus 55.9%, $P = 0.007$).

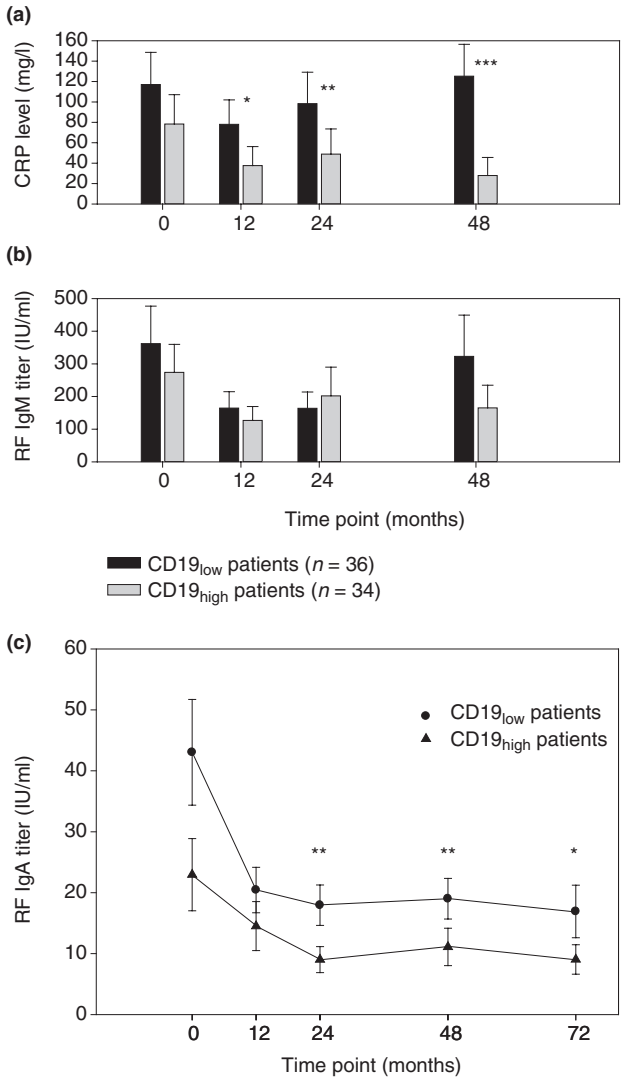
Separation of SE-positive patients according to the expression of the shared epitope either on a DR4 or a DR1 allele showed significantly lower numbers of circulating B cells in both groups when compared with SE-negative patients (93.845 versus 163.7; interquartile range, 6.7–177.1 versus 117.4–243.4 [$P < 0.05$] for SE $DR4^+$ patients; and 101.2 versus 163.7; interquartile range, 48.4–147.0 versus 117.4–243.4 [$P < 0.05$] for SE $DR1^+$ patients) (see Fig. 2). While a significant correlation was found between absolute B-cell counts and T-cell counts, no difference in the number of circulating $CD4^+$ T cells was discerned between SE-positive and SE-negative patients (for details, see Supplementary material).

Characterization of patients with diminished numbers of $CD19^+$ B cells

Analysis of the C-reactive protein (CRP) values determined simultaneously with the B-cell numbers in the prospective analysis revealed that $B\text{-cell}_{low}$ patients had higher median CRP levels (9.3 mg/l versus 5.2 mg/l, $P < 0.05$). In addition, the analysis of the prospectively documented values at study entry and after 1 year of observation showed a trend for higher CRP levels in the $B\text{-cell}_{low}$ group (median, 24.4 mg/l versus 9.2 mg/l [$P = 0.09$], and 10.6 mg/l versus 5.0 mg/l [$P = 0.06$], respectively) that reached significance after 2 and 4 years of observation (median, 16.4 mg/l versus 5.0 mg/l [$P = 0.01$], and 14.0 mg/l versus 5.4 mg/l [$P = 0.01$], respectively) (see Fig. 3a).

The $CD19_{low}$ group of patients did not show a higher frequency of RF IgM seropositivity or higher RF IgM titers (Fig. 3b). $CD19_{low}$ patients were characterized, however, by higher RF IgA titers after 1, 2 and 4 years of observation in the prospective study (median, 40.0 IU/ml versus 0 IU/ml [$P < 0.02$], 33.0 IU/ml versus 0 IU/ml [$P < 0.01$],

Figure 3



Comparison of **(a)** C-reactive protein (CRP) levels, **(b)** rheumatoid factor (RF) IgM titers, and **(c)** RF IgA titers in patients below ($CD19_{low}$) and above ($CD19_{high}$) the mean of the study population (110 B cells/ml), which was determined after a mean disease of 4.4 years. The different time points of observation are indicated on the *x* axis, starting from the first visit in the rheumatology clinic. All graphs depict the mean and standard error of the mean. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

and 63.5 IU/ml versus 0 IU/ml [$P < 0.001$], respectively) (Fig. 3c). Analysis of differential blood counts obtained from all patients simultaneously with the determination of absolute cell number showed $CD19_{low}$ patients to have fewer lymphocytes (median, 1.06×10^6/l versus 1.60×10^6/l, $P = 0.001$), while no differences in monocyte number were discerned (median, 0.49×10^6/l versus 0.48×10^6/l, $P = 0.77$).

A detailed analysis of DMARD usage in patients below and above the mean of the study population (110 cells/ml, $CD19_{low}$ and $CD19_{high}$ patients, respectively) importantly revealed no significant differences between the two groups (see Table 2).

Discussion

The influence of immunogenetic parameters on the course of RA has been explored by a number of prospective studies [22,27–30]. In several Caucasian study populations, patients positive for RA-associated DRB1 alleles, and in particular those expressing the so-called shared epitope on a DRB1*04 allele, were found to suffer from a more rapid and severe course of joint destruction. With regards to RF production, one copy of the shared epitope seems sufficient to transmit a significantly increased risk for the development of RF IgM-positive RA [31].

A predominant role for B-cell activation and autoreactive humoral responses has been invoked not only for human RA, but also for many animal arthritis models. Immunoglobulins are crucial for the classical collagen-induced arthritis [13], while the recently published K/BxN mouse system absolutely requires autoreactive B cells for the erosive arthritis to develop [32]. B-cell activation by newly described stimulatory interactions between the B-cell surface receptor B lymphocyte stimulator (BlyS) and the transmembrane activator and CAML interactor (TACI) [33] has also recently been reported to be required for the induction of collagen-induced arthritis in rodents [34].

Our chief finding of a significant influence of the RA-associated shared epitope on the numbers of circulating B cells in RA patients has not been reported previously. Several different explanations for this phenomenon are feasible, none of which can be ruled out at present.

Since SE-positive RA is generally regarded as a more severe disease, it can be hypothesized that high numbers of involved lymphocytes, including B cells, are consumed in the long-standing autoimmune response in SE-positive RA patients. This is contradicted, however, by the lack of association of diminished B-cell numbers with prolonged disease duration or with increased DMARD therapy, or a more rapid joint destruction found in both study cohorts.

In view of animal experiments demonstrating clonal deletion of RF-producing B cells on encounter of their antigen [35], decreased absolute B-cell numbers could reflect a substantial loss of B cells in SE-positive RA. It can be hypothesized that this loss is accompanied by repertoire contraction and oligoclonality in the B-cell compartment of RA patients, which parallels T-cell repertoire changes found in RA [36].

In a recent study, widespread clonal expansion could be shown in B cells from peripheral blood and synovial membranes from patients with RA [37]. The immunoglobulin V_H

Table 2

Disease-modifying antirheumatic drug usage in $CD19_{high}$ and $CD19_{low}$ patients in the prospective study cohort

	$CD19_{high}$ patients ($n = 33$)	$CD19_{low}$ patients ($n = 37$)
Disease duration (years)	6.54	6.68
MTX at time of analysis [*n* (%)]	26 (78.8)	30 (81.1)
MTX dose (mg) [mean (range)]	15.4 (15–20)	16.1 (15–20)
MTX in combination with cyclosporine A	2	3
Duration of MTX therapy (months)	40.1	43.3
MTX-treated patients positive for SE [*n* (%)]	22 (66.7)	19 (51.3)
Tauredon therapy	1	2
Cyclosporine A	1	1
Chloroquine	2	0
Prednisolone at time of analysis (mg)	4.8	4.8
Dose range (mg)	3–10	3–7

MTX, methotrexate; SE, DRB1 shared epitope. Comparison of patients below and above the mean of the study population (110 cells/ml, $CD19_{high}$ and $CD19_{low}$ patients, respectively) of the prospective study cohort. The number of patients receiving the indicated disease-modifying antirheumatic drug and the dose ranges are presented. None of the comparisons show statistically significant differences.

gene fingerprinting assay used in that study allowed the discrimination of numerically expanded B-cell specificities from merely activated clones. The detected numerical clonal expansions could therefore be indications for a restricted repertoire of B lymphocytes in RA, which parallels the B lymphocytopenia described in the present study and is likely to be the consequence of the disturbed B-cell homeostasis in RA. The primary mechanism driving those B-cell repertoire aberrations is likely to act in the synovial membranes of synovitic joints, since clonality is more pronounced there [37] and the frequencies of B cells specific for relevant autoantigens that have already undergone the isotype class switch to IgG/IgA are higher among synovial B cells [38]. Taken together, these repertoire studies indicate that clonal growth and depletion, possibly in the context of MHC-restricted T-cell help [39], might be a regulatory factor in B-cell homeostasis in RA.

Alternatively, since only a small fraction of the total B-cell pool is found in the peripheral circulation, diminished numbers of circulating $CD19^+$ B cells might be the result of increased accumulation of autoreactive B cells in the synovial membrane of affected joints. Irrespective of the underlying mechanisms, the association of diminished numbers of circulating $CD19^+$ B cells with increased disease activity in the prospective study population indicates that an absolute B-cell count might be used as an additional, readily available clinical parameter. Whether this parameter is of clinical relevance and possibly might be used as a prognostic or response indicator needs to be explored in further prospective studies.

Conclusion

The results presented indicate a profound influence of the presence of RA-associated immunogenetic parameters on B-cell homeostasis in RA. The decreased numbers of circulating $CD19^+$ B lymphocytes that are present in SE-positive patients are associated with increased disease activity and RF IgA production. DMARD usage or the pace of joint destruction, however, did not have an influence on B-cell homeostasis.

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Correspondence

Holm Häntzschel, Department of Medicine IV, University of Leipzig, Härtelstraße 16–18, 04107 Leipzig, Germany. Tel: +49 341 97 24700; fax: +49 341 97 24729; e-mail: haentzho@medizin.uni-leipzig.de

Supplementary material

Supplementary Materials and methods

Serum concentrations of RF IgM and IgA were determined using a standard ELISA assay (Autozyme™ RF; Cambridge Life Sciences, Cambridge, UK). The normal range

Supplementary Table 1

Absolute number of cells per milliliter of blood

	$CD4^+$	$CD4^+CD45RA^+$	Lymphocytes	Monocytes
CD19+				
R	**0.422**	0.233	**0.512**	−0.098
P	**0.0003**	0.054	**< 0.0001**	0.445
CD4+				
P		**0.808**	**0.677**	0.061
R		**< 0.0001**	**< 0.0001**	0.634

Data presented as correlation coefficient (*R*) and level of significance (*P*). Significant correlations in bold.

in this assay, as given by the manufacturer and confirmed from the central laboratory facility at our institution, is below 40 IU/ml. Titers of antinuclear factors were determined on Hep2 cells (Euroimmun, Mosaic Hep2/liver slides, Lübeck, Germany) in serial serum dilutions starting at a sample dilution of 1:40. For quantification of antibodies against double-stranded DNA, a commercial ELISA system was used (VarELISA; Pharmacia Upjohn, Erlangen, Germany). Serum concentrations of IgM, IgG and IgA were determined by a nephelometric assay on BN 2 (Dade Behring, Schwalbach, Germany) using N antisera to IgM, IgG and IgA (Dade Behring). Concentrations of circulating immune complexes were also determined by a nephelometric test (Dade Behring).

For flow cytometry, peripheral blood mononuclear cells were separated using Ficoll density gradient centrifugation, and were then incubated for 20 min at 4°C with the following antibodies (Becton Dickinson, San Jose, CA, USA): anti-CD4 FITC, anti-CD8 FITC, anti-CD3 PE, anti-CD19 FITC, and the antibody combination anti-CD45RA FITC/CD4 PE. Samples were washed and analyzed on a FACS Calibur (Becton Dickinson, Heidelberg, Germany).

For HLA DRB1 genotyping, cellular DNA was isolated from 10 ml peripheral blood using standard procedures, and 0.5 µg DNA were used in a PCR with two primers specific for the second exon of DRB1, as described previously [27]. Low-resolution typing of DRB1 specificities was performed by oligonucleotide hybridization of the PCR products to probes specific for DRB1*01 through DRB1*18 (for a complete listing of primers and probes, see [32]). Hybridization was performed in a dot-blot format with digoxigenin-11-ddUTP-labeled oligonucleotides. After the stringent wash, detection was carried out using anti-digoxigenin antibody–alkaline phosphatase conjugate (Boehringer Mannheim, Mannheim, Germany) and disodium 3-(4-methoxyspiro(1,2-dioxetane-3,2-(5′-chloro)tricyclo[3.3.1.13,7]decan)-4-yl)phenyl phosphate (Tropix, Bedford, MA, USA) as the chemiluminescent substrate. For DRB1*04 subtyping, primers and oligonucleotides were again used as published previously [27].

Statistical analysis was performed using the software package SigmaStat for windows (SPSS Inc., Chicago, IL, USA). The distributions of frequencies of $CD19^+$ B cells in RA patients were analyzed using the Kolmogorov–Smirnov two-sample test. For all other comparisons, Student's *t* test or the Mann–Whitney rank sum test was used where appropriate. For correlation analysis, the Spearman rank order correlation test or the Pearson product moment correlation test was used depending on the data distribution.

Supplementary Results

Descriptive analysis of lymphocyte subpopulations

In the prospectively followed patient group, absolute numbers of $CD4^+$ T cells and of $CD4^+CD45RA^+$-naive T cells were determined in parallel to the $CD19^+$ B cells using the TRUCOUNT® technology. In addition, the total lymphocyte and monocyte counts were obtained by conventional differential blood count. Correlation analysis of the absolute cell counts revealed significant correlations between the different lymphocyte subpopulations, while the absolute numbers of monocytes appeared not to be related.

The total number of lymphocytes obtained from the patients' differential blood counts showed a significant correlation with the absolute number of $CD19^+$ B cells, but also with $CD4^+$ T cells. The correlation coefficient for the latter was markedly higher. Furthermore, $CD19^+$ B-cell counts were not related to the number of naive or memory T cells, while total $CD4^+$ T-cell counts and naive T-cell counts correlated very closely. Results of the correlation analyses are presented in Supplementary Table 1.

Clinical description of $CD19_{low}$ and $CD19_{high}$ patients

The $CD19_{low}$ group of patients and the $CD19_{high}$ group of patients were compared in both the retrospective study cohort and the prospective study cohort with regards to the clinical parameters of their disease (Supplementary Table 2). In the retrospective study group, no significant differences were discerned in the laboratory findings of the $CD19_{low}$ and $CD19_{high}$ groups, while the differences in CRP and RF IgA levels found in the prospective study are depicted in Figure 3.

Radiological findings in $CD19_{low}$ and $CD19_{high}$ patients

In the retrospective study group, the degree of joint destruction was determined on the last available radiograph of the hands. As a parameter applicable to the advanced stage of joint destruction present in the majority of cases, radiographs were analyzed for the presence of fibrous or bony ankylosis of digital joints or wrists. Of the retrospective study group patients, 40.7% had evidence

Supplementary Table 2

Comparison of $CD19_{high}$ patients and $CD19_{low}$ patients of the retrospective study group cohort and the prospective study group cohort

	$CD19_{low}$ group	$CD19_{high}$ group	Level of significance
Retrospective study group	$n = 58$	$n = 36$	
Age (years)	63.8 (56–72)	62.7 (56–72)	0.613
Disease duration (years)	11.4 (7.1–22.6)	19 (5.8–27.7)	0.446
ESR (mm/h)	35.9 (22.83–3.19)	31.52 (21.72–3.9)	0.393
C-reactive protein (mg/l)	27.6-(6.5–60)	15.7 (7.18–40.28	0.204
Serum IgM (g/l)	1.65 (1.20–2.26)	1.92 (1.60–2.61)	0.022
Serum IgG (g/l)	10.45 (9.09–11.70)	10.50 (9.54–13.00)	0.498
Serum IgA (g/l)	2.67 (1.89–3.73)	2.67 (1.88–3.91)	0.724
RF IgM (IU/ml)	134.8 (84–257)	187 (110–207)	0.182
RF IgA 9IU/ml)	75 (33.5–128.75)	31.5 (13.5–106.5)	0.02
ANF (titer)	1:160 (0–1:320)	1:160 (0–1:1120)	0.861
Circulating immune complexes(g/l)	4.6 (3.32–9.15)	4.45 (3–5.8)	0.607
Prospective study group	$n = 36$	$n = 34$	
C-reactive protein (mg/l)	14 (5–25.5)	5.4 (0–9.2)	0.013
Serum IgM (g/l)	1.07 (0.79–1.4)	1.19 (0.88–1.43)	0.618
Serum IgG (g/l)	11.5 (10.22–13.7)	12.7 (11–14.2)	0.371
Serum IgA (g/l)	3.08 (2.04–3.86)	2.35 (1.78–3.02)	0.062
RF IgM (IU/ml)	132 (31.1–253)	65.5 (0–136)	0.099
RF IgA (IU/ml)	63.5 (12–155.8)	0 (0–8)	< 0.001

ANF, antinuclear factors; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor. Data are presented as medians (interquartile ranges) of all parameters, and the resulting level of significance are given.

of fibrous or bony ankylosis in their hand radiographs, indicating the advanced stage of disease. The more aggressive course of joint destruction in SE DR4-positive patients was confirmed by the high percentage of those patients (58.3%) with ankylosing changes in hand radiographs, compared with only 25% of SE DR4-negative patients ($P = 0.007$). With regards to the percentage of $CD19^+$ B cells, no different radiographic outcome was evident since 40.4% of $CD19_{low}$ patients and 41.4% of $CD19_{high}$ patients had radiographic evidence of ankylotic joints ($P = 0.87$).

For the prospective study group, serial radiographs had been taken every 6 months and scored according to Larsen's method as described previously [27]. No significant differences were seen between the $CD19_{low}$ patient group and the $CD19_{high}$ patient group after 2 or 4 years of observation (median Larsen score, 20 versus 24 [$P = 0.29$] after 2 years of observation, and 29 versus 26 [$P = 0.55$] after 4 years of observation).