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The Th2 Lymphoproliferation Developing in LatY136F Mutant Mice Triggers Polyclonal B Cell Activation and Systemic Autoimmunity

Céline Genton,* Ying Wang,† Shozo Izui,‡ Bernard Malissen,† Georges Delsol,§¶ Marie Malissen,2† and Hans Acha-Orbea2, 3*

LatY136F knock-in mice harbor a point mutation in Tyr136 of the linker for activation of T cells and show accumulation of Th2 effector cells and IgG1 and IgE hypergammaglobulinemia. B cell activation is not a direct effect of the mutation on B cells since in the absence of T cells, mutant B cells do not show an activated phenotype. After adoptive transfer of linker for activation of T cell mutant T cells into wild-type, T cell-deficient recipients, recipient B cells become activated. We show in vivo and in vitro that the LatY136F mutation promotes T cell-dependent B cell activation leading to germinal center, memory, and plasma cell formation even in an MHC class II-independent manner. All the plasma and memory B cell populations found in physiological T cell-dependent B cell responses are found. Characterization of the abundant plasmablasts found in secondary lymphoid organs of LatY136F mice revealed the presence of a previously uncharacterized CD93-expressing subpopulation, whose presence was confirmed in wild-type mice after immunization. In LatY136F mice, B cell activation was polyclonal and not Ag-driven because the increase in serum IgG1 and IgE concentrations involved Abs and autoantibodies with different specificities equally. Although the noncomplement-fixing IgG1 and IgE are the only isotypes significantly increased in LatY136F serum, we observed early-onset systemic autoimmunity with nephritis showing IgE autoantibody deposits and severe proteinuria. These results show that Th2 cells developing in LatY136F mice can trigger polyclonal B cell activation and thereby lead to systemic autoimmune disease. The Journal of Immunology, 2006, 177: 2285–2293.

Systemic autoimmune disease is often associated with autoantibody production. Systemic lupus erythematosus, for example, is a chronic autoimmune condition that results in inflammation and kidney damage. Many disease susceptibility genes are involved, and several of these affect survival and activation of B lymphocytes resulting in increased autoantibody production (1, 2). Autoantibodies directed toward cellular and extracellular matrix components such as DNA, chromatin, cell membrane, and matrix play an important role in the development of nephritis, and deposition of immune complexes in kidney glomeruli is part of the disease process (for review, see Ref. 3). Abs with (cross)-reactivity to glomerular membrane proteins have been suggested to be of importance (4, 5). Several excellent mouse models of spontaneous nephritis exist. Models with germinal center (GC)* formation, affinity maturation, and preferential amplification of autoantibody-producing plasma cells, as well as models with polyclonal activation of B cells, resulting in similar disease patterns, have been described previously (6–8).

During T-dependent immune responses, Ag-specific, naïve T cells are primed by Ag-presenting dendritic cells (DC) (9). B cells recognizing native Ag through their surface Ig are activated in parallel in the follicles and preferentially localize to the follicular regions of secondary lymphoid organs (10–12). There, they receive T cell help if they are able to present to the primed T cells the same peptides that were presented by DC during T cell priming (11, 13). Interaction between T and B cells constitutes a key event in both the formation of extrafollicular plasmablasts producing IgM or switched isotypes and in the initiation of the GC reaction in the follicular regions of secondary lymphoid organs (14). In GC, isotype switching, affinity maturation, and differentiation into short- or long-lived plasma cells or memory B cells are achieved with the help of follicular DC (FDC). After having recapitulated the (same) priming Ag from FDC, apoptosis-sensitive centrocytes receive T cell help from follicular T cells (14). These steps ensure survival and maturation of B cells that have maintained Ag specificity and optimized their affinity for Ag (15).

Abbreviations used in this paper: GC, germinal center; BAFF, B cell-activating factor belonging to the TNF family; DC, dendritic cell; FDC, follicular dendritic cell; LAT, linker for activation of T cell; PNA, peanut lectin (agglutinin); WT, wild type; NP, 4-hydroxy-3-nitrophénylacétate; PALS, periarteriolar lymphoid sheath; TNP, 2,4,6-trinitrophenyle.
Physiological T cell-B cell interactions are sometimes not as specific as might be expected from the above description. It has been observed that only ~10% of plasma cells secrete Abs specific for the encountered Ags during an immune response induced by different protein Ags such as sheep RBC (16). Both Ag-independent polyclonal B cell activation by Ag-primed T cells and Ag-dependent T cell help to B cells that have captured Ag through other mechanisms than uptake via surface Ig have been described previously (17). In this latter mode of “unspecific” T-B cell help, the amounts of Ag required are ~10,000 times higher than those required for T-B interactions involving B cells expressing an Ag-specific Ig (17, 18).

Linker for activation of T cell (LAT) is an adaptor protein that constitutes a major substrate for the Zap70 protein tyrosine kinase in T cells (19). Once phosphorylated, LAT coordinates the assembly of a multiprotein signaling complex that links the TCR to the main intracellular pathways regulating T cell development and function. LatY136F knock-in mice carry a mutation in LAT that replaces Tyr at position 136 with phenylalanine and principally eliminates binding of phospholipase C-γ1. Mutation of Tyr136 results in a partial block at the two developmental checkpoints that punctuate intrathymic αβ T cell development (20, 21). However, beginning at ~2–3 wk of age, LatY136F mice develop a lymphoproliferative disorder involving polyclonal CD4 T cells that produce high amounts of Th2 cytokines. This exaggerated Th2 differentiation is most likely responsible for the massive activation of B cells and the hyperggammaglobulinemia of IgE and IgG1 isotypes that ensues.

In this study, we show that the polyclonal B cell activation in LatY136F mice is not due to a direct (intrinsic) effect of the LAT mutation on B cells but indirectly results from the presence of CD4 Th2 effector cells. This polyclonal B cell activation involves the formation of abundant GC and leads to the formation of abnormally large numbers of memory B cells, plasmablasts, and plasma cells. The exaggerated B cell differentiation occurring in LatY136F mice allowed the characterization of a novel CD93-positive plasmablast subpopulation that was also found in normal B cell responses. The GC and plasma cell populations found in LatY136F mice are phenotypically comparable to those found in physiological immune responses. Surprisingly, the B cell help provided by the LatY136F CD4 T cells was independent of TCR-MHC interaction, as documented by the fact that LatY136F CD4 T cells induced MHC class II-negative B cells to secrete Ab in vitro as efficiently as MHC class II-expressing B cells. In vivo, adoptive transfer of LatY136F T cells resulted in reduced, but clearly detectable, MHC class II-independent GC formation and polyclonal Ab secretion. The Th2-mediated polyclonal B cell activation observed in LatY136F mice resulted in early-onset systemic autoimmune disease with nephritis. The increase in autoantibodies observed in LatY136F mice was not Ag driven but was proportional to the increase in total Ig. Based on their IgG1 and IgE isotypes, the autoantibodies present in LatY136F mice were expected to be less pathogenic than complement-fixing autoantibodies. However, nephritis with IgE and in ~30% of cases complement deposits in the glomeruli, increased serum urea levels, as well as proteinuria, were readily observed in young LatY136F mice.

Materials and Methods

Mice

LatY136F mice (20), Cδ3-eG2A55 mice (22), and Cδ3-eG2A55 × LatY136F mice have been obtained from the Centre d’Immunologie de Marseille-Luminy and were backcrossed for more than six generations to the C57BL/6 background. MHC class II−/− mice (H2–/−) were a gift from W. Reith (Centre Médical Universitaire, Geneva, Switzerland) (23). All mice were maintained at the Swiss Institute for Experimental Cancer Research. All animal experiments were done in agreement with Institutional and Swiss regulations.

Immunohistological analysis

Studies were performed on acetone-fixed frozen sections and stained using standard methods. The following reagents were used: biotinylated anti-B20 (RA3-682; Caltag Laboratories), anti-CD4 (H129) culture supernatant, biotinylated peanut lectin (agglutinin) (PNA) (Vector Laboratories), biotinylated anti-IgGl (Caltag Laboratories), biotinylated anti-IgE (University of Louvain), and goat anti-C3 (Cappel Laboratories). Biotinylated Abs were visualized with HRP-conjugated streptavidin (Jackson ImmunoResearch Europe), except for the double staining where anti-B20 was detected with alkaline phosphate-conjugated streptavidin (Boehringer Mannheim). Nonconjugated supernatant was detected using HRP-conjugated goat-anti-rat Ab (BioSource International). Goat Abs were detected using HRP-conjugated goat-anti-rat Ab (Jackson ImmunoResearch Europe) (24) or using a secondary anti-IgG Abs (Invitrogen Life Technologies). Histopathologic examination was performed on 12 LatY136F mice and 4 wild-type (WT) mice. All major organs (spleen, thymus, lymph node, lung, and kidney) were fixed in neutral buffered 4% Formalin. Paraflin-embedded sections were stained with H&E and with Giemsa. Immunohistochemistry was performed with CNA.42 Ab directed against FDC (24). After incubation with the aforementioned Abs, sections were imnuno-stained with biotinylated streptavidin complex method using DacoCytometry StrepABC/HRP Duet (Mouse/Rabbit) Kit (code K0492; DacoCytometry) as described elsewhere (25). Immunostaining on paraffin sections was performed using the method described by Shi et al. (26) with some modifications (25).

Flow cytometry

Single-cell suspensions were stained using standard techniques. mAbs used for flow cytometry were B220 (RA3-682; BioSource International), MHC class II (2G9; BD Pharmingen); syndecan-1 (281-2; BD Pharmingen); GL-7 (Ly77; BD Pharmingen); PNA (Sigma-Aldrich), CD4 (GK1.5; BD Pharmingen); CD8 (53.6-7; Biolegend); CD93 (PB493; BD Pharmingen); and IgD (11-26c.2a). Biotinylated Abs were visualized with streptavidin-allophycocyanin (Caltag Laboratories) and streptavidin/PE/Cy5.5 (BD Bioscience). Culture supernatant 2.4G2 has been used to block Fc receptors. Flow cytometry was performed on a FACScan flow cytometer. Antibody was performed on a live gate based on forward and side scatter characteristics using CellQuest software 3.2.1f1 (BD Pharmingen) and FlowJo 6.2.1.

Analysis of Ab titers

Serum levels of total IgM, IgG1, IgG2a, IgE, and 4-hydroxy-3-nitrophenylacette (NP)-specific IgG1 Abs were quantified by ELISA using polyclonal goat Abs specific for mouse Ig isotypes for detection (Caltag Laboratories) and o-phenylenediamine developing reagents (Sigma-Aldrich). To detect anti-NP Abs, plates were precoated with NP (23)-BSA (BioSource International). For quantifying serum urea, the kit from Sigma-Aldrich was used.

In vitro coculture assay

CD4+ cells were first enriched by MACS (Miltenyi Biotec) using anti-CD4 beads and then sorted by FACS for maximal purity (at least 99%). A total of 3 × 105 CD4+ cells was cultured with 9 × 104 WT or MHC class II−/− splenocytes in 200 μl of DMEM (Invitrogen Life Technologies) with 10% FCS (Biosera), 10 mM HEPES buffer (Invitrogen Life Technologies), 200 μg/ml gentamicin (Invitrogen Life Technologies), and 50 μM 2-ME (Invitrogen Life Technologies). IgG1 was quantified in culture supernatant by ELISA at different time points. Concentration of IgG1 was determined by comparing a test sample dilution series with that using a control IgG1 standard (BD Pharmingen).

Adaptive transfer

Single-cell suspensions of spleen and lymph nodes from 5- to 8-wk-old mice were enriched for CD4+ T cells using magnetic beads conjugated with CD4 (Miltenyi Biotec) on an AutoMACS. A total of 3 × 105 CD4+ T cells (purity > 95%) from the specified mice was injected i.v. into 4- to 6-wk-old CD3-eG2A55 mice or CD3-eG2A55 × IgE−/− mice. Mice
were treated with Bactrim and maintained in specific pathogen-free conditions for 8 wk before analysis.

Autoimmune disease analysis

Serum levels of IgG and IgE autoantibodies against chromatin and DNA were determined by ELISA as described previously (27, 28). Results are expressed either as units per milliliter, in reference to a standard curve derived from a serum pool of MRL-Faslpr mice, or as OD405. A flow cytometric assay was used to detect Coombs’ anti-erythrocyte autoantibodies using PE-labeled anti-mouse κ L chain Abs (BD Pharmingen), as described previously (29). The results are expressed as mean fluorescence intensity. The presence of proteinuria was measured in a semiquantitative way using Albustix (Bayer). Mice were scored as positive for proteinuria when protein levels exceeded 300 mg/dl defined by the colorimetric test for two measures at an interval of at least 1 wk. Paraformaldehyde-fixed kidney histological sections were stained with periodic acid-Schiff reagent. The extent of glomerulonephritis was graded on a scale of 0 – 4 based on the intensity and extent of histological changes, as described previously (30). The scoring was performed in a double-blind mode. Ab deposits in the kidney were detected using anti-IgG1 and anti-IgE staining of kidney sections.

Statistical analysis

Statistical analysis was performed with the Mann-Whitney rank-sum test to compare WT and LatY136F mouse autoantibody level, Ig fold increase, kidney disease index, and for in vitro assay. The Fisher exact test has been used to determine significant differences between WT and LatY136F proteinuria analysis. Values of $p < 0.05$ were determined as significant for each of the tests.

Results

Status of the B cell compartment in LatY136F mice

Adult LatY136F mice show serum IgG1 and IgE concentrations that are elevated 200 and 10,000 times, respectively, compared with WT mice (20, 21). Histopathologic examination of the spleen showed white pulp hyperplasia. Periarteriolar lymphoid sheaths (PALS) were pale with a striking accumulation of plasma cells associated with plasmablasts and large immunoblast-like cells (Fig. 1, A (control) and B (LatY136F)). Variable numbers of eosinophils and basophils/mast cells were found. Lymph nodes were

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enlarged and showed variable hyperplasia of lymphoid fociles and an accumulation of cells with morphologic features comparable to that observed in the spleen.

Analysis of the development of this pathologic phenotype showed that until 2 wk after birth, most B lymphocytes had a naive phenotype (data not shown). At this age, we observed a normal distribution of B cells in lymphoid fociles but almost a complete absence of CD4 T cells in the paracortex and PALS (Fig. 1C and data not shown). FACS analysis confirmed these results and showed that in 2-wk-old LatY136F mice there were eight times fewer CD4 T cells in lymph nodes than in littermate controls (Fig. 1D). As previously described (20, 21), there were few CD8 T cells in LatY136F mice at all ages examined.

In secondary lymphoid organs of 4-wk-old LatY136F mice, CD4 T cell numbers increased and were mostly localized to the paracortex and the PALS. Coincident with this increase in CD4 T cells, large PNA− GC appeared in every lymph node and spleen B cell follicle (Fig. 1, C and D, and data not shown). In addition to activated B and T cells, both lymph nodes and spleen contained large quantities of Ab-secreting (B220+/−, MHC class II+; syndecan-1−) plasmablasts (Fig. 1D). In 6-wk-old LatY136F mice, Ab-secreting plasmablasts/plasma cells (as defined by detecting B220 down-regulation and syndecan-1 expression) reached 5% of total lymph node cells. Considering that lymph nodes of 6-wk-old LatY136F mice are five times bigger than those of WT controls, 0.6 × 10^6 Ab-secreting cells, 2.3 × 10^6 GC cells, and 6 × 10^6 T cells could be isolated from a single LatY136F lymph node (Fig. 1D). This represents an ~500-fold increase in plasmablasts/plasma cell numbers compared with either age-matched, heterozygous littermate controls or to WT mice. Analysis of LatY136F mice showed that lymph node activation became more severe with time, leading to a complete disruption of lymphoid follicle architecture by 3 mo of age (data not shown). Thus, there was a clear correlation between the increase in splenic and lymph node CD4 T cell number, Th2 phenotype, and the activation status of B cells.

The LatY136F mutation acts indirectly on B cells

LAT is expressed during early stages of B cell differentiation at node cells. Considering that lymph nodes of 6-wk-old LatY136F mice were isolated from a single

FIGURE 3. Plasma cell compartment in LatY136F lymph node. A, Flow cytometric analysis of plasma cells in lymph node from 6-wk-old WT and 2-, 4-, and 6-wk-old LatY136F mice. Syndecan-1− cells are shown as black dots on the B220/MHC class II graph. B, Percentage of syndecan-1− among B220− MHC class II+ cells in the lymph node. WT mice (filled) are compared with LatY136F mice (black line). C, CD93 expression by syndecan-1− cells in lymph node from 2-mo-old LatY136F mice.

Th2-mediated systemic autoimmunity in LatY136F mice

To examine whether the aberrant CD4 T cells developing in LatY136F mice activate B cells polyclonally or primarily activate autoreactive B cells, we compared the relative titers of anti-NP, affected later stages of B cell activation and differentiation, we generated double-mutant mice deficient for both CD3ε polypeptides (CD3ε−/−) (22) and the LatY136F mutation. In these double-mutant mice, direct activation of B cells via T cell help can be excluded because they lack both αβ and γδ T cells. FACS analysis of developing B cells in the bone marrow and of the maturation and activation status of mature B cells in the spleen and lymph nodes of CD3ε−/− × LatY136F double-mutant mice showed no differences compared with double heterozygous or WT littermate controls (data not shown). Histological analysis of lymph nodes from double-mutant mice showed no effect on B cell localization or activation, and no GC developed in 3-mo-old mice (Fig. 2, A and B). FACS analysis showed that most B220− cells had a naive phenotype as in WT mice (Fig. 2B). When CD3ε− × LatY136F mice were compared with WT and CD3ε−/− mice, they showed no increase in either GC B cell populations or in Ab-secreting cells (Fig. 2B). Moreover, there was no B cell lymphoproliferation in CD3ε− × LatY136F mice; their lymph node cellularity was similar to that of CD3ε−/− mice. Their serum levels of IgG1 and IgE were also comparable to those of CD3ε−/− mice (Fig. 2C). Therefore, the massive B cell activation observed in young LatY136F mice was not due to a direct effect of the LatY136F point mutation on B cells but was clearly T cell dependent.

Phenotype of GC and Ab-secreting cells in LatY136F mice

To compare the phenotype of activated and differentiated B cells in LatY136F mice, we analyzed their surface phenotype. All memory and effector B cell populations that characterize a primary carrier-hapten-specific immune response were found in LatY136F mice (Fig. 3, A and B, and data not shown). Surprisingly, a prominent proportion of plasmablasts/plasma cells expressed CD93, a previously unrecognized marker of this population (Fig. 3C). The same population can also be found in physiological immune responses (data not shown). Therefore, the B cell subpopulations seen in LatY136F mice are comparable to those found in physiological immune responses, differing in the strongly increased number in the LatY136F mice.
anti-2,4,6-trinitrophenyl (TNP) Abs, and autoantibodies in nonimmunized LatY136F mice with those of heterozygous littermate controls (see Table I).

Total Ig serum titers were increased 30-fold in 3-mo-old LatY136F mice. Among the predominant IgG1, which were increased 158-fold, anti-NP-Abs were increased 20-fold and anti-TNP 100-fold. Autoantibodies directed against DNA were increased 100-fold. Other autoantibodies directed at cytokeratin and laminin, as well as Abs to control proteins such as chicken gammaglobulin, showed low to undetectable reactivity in WT mice but, when detectable in controls, were increased proportional to the total Ig increase. Proportionality for IgE could not be measured as normal mice had very low serum IgE levels. Taken together, these results indicate that LatY136F mice develop polyclonal B cell activation without a bias for B cells secreting autoantibodies.

We next tested whether the help provided to B cells by LatY136F CD4 T cells required interaction between TCR and MHC class II molecules. For this purpose, we first mixed FACS-sorted LatY136F CD4 T cells with WT B cells and measured in vitro the induction of IgG1 production by WT B cells (Fig. 4A). When WT B cells were cultured in the presence of LatY136F CD4 T cells, IgG1 Abs were easily detectable after 6 days, and levels further increased with time. Purified WT B cells together with WT CD4 T or sorted CD4 T cells from LatY136F mice did not induce production of detectable amounts of IgG1. Based on our observation that blocking Abs to β2 integrin completely prevented IgG1 secretion, we conclude that B cell activation is contact dependent. In addition, blocking IL-4 with mAbs prevented IgG1 secretion (data not shown). When B cells lacking MHC class II expression (I-Aα−/−) were incubated in the presence of LatY136F CD4 T cells, they produced levels of IgG1 similar to WT B cells (Fig. 4B). In control experiments with WT CD4 T cells and WT B cells, no induction of Ab secretion was detected during the coculture period (data not shown). Therefore, consistent with the poor signaling properties of the TCR expressed on LatY136F CD4 T cells (20, 21), the fact that help delivered to B cells by LatY136F CD4 T cells does not rely on interaction between the TCR and MHC class II molecules likely accounts for the production of an unbiased IgG1 and IgE repertoire.

To analyze this point further, we adoptively transferred purified LatY136F CD4 T cells into Cd3−eΔ5/Δ5 mice that were MHC class II expressing or deficient. In both instances, clear induction of GC was observed (Fig. 4C). However, increases in Ab titers were only slightly delayed in MHC class II-deficient recipients. Taken together, these results suggest that the B cells found in LatY136F mice are activated by polyclonal T cell help and that MHC class II expression is not required for induction of B cell activation, formation of GC centers, isotype switch, or Ab secretion.

LatY136F mice develop autoantibodies and a systemic autoimmune syndrome with involvement of kidney, lung, and liver

LatY136F mice have elevated total IgG1 and IgE serum titers and anti-nuclear Abs (20, 21). Results shown in Table I and Fig. 5, A and B, illustrate that B cells undergo polyclonal activation without

**FIGURE 4.** Independence on TCR-MHC interaction for B cell activation by LatY136F CD4+ T cells. A, Total WT splenocytes were cultured in the presence or absence of FACS-sorted CD4+ LatY136F cells. B cell activation and differentiation was monitored by measuring IgG1 production in culture supernatant by ELISA after 2, 6, 12, and 18 days of culture. Purified CD4+ LatY136F cells or WT splenocytes alone were used as negative controls. B, Splenocytes from MHC class II-deficient mice were used in the same assay in the presence (■) or absence (□) of CD4+ LatY136F cells. Data are representative of at least three independent experiments. C, CD4+ cells were transferred into a CD3−eΔ5/Δ5 recipient. Histological analyses were performed on spleen sections. Anti-B220 (blue) and anti-CD4 (brown) staining shows the T and B cell distribution. PNA (brown) staining indicates GC formation. Nuclei are counterstained with Mayer’s hematoxylin (blue). Significant differences as calculated by the Mann-Whitney rank-sum test are indicated. **, p < 0.01.
Strikingly, the FDC net-scattered large immunoblasts (Fig. 7 in infiltrates consisting of small lymphoid cells, plasma cells, and showed striking alterations. The most interesting changes were ob-

E later time point (Fig. 6). By measuring the titers of anti-erythrocyte Abs (Fig. 5B), anti-laminin (Table I), anti-chromatin (Fig. 5B), and anti-DNA Abs (Fig. 5C), we observed that LatY136F mice had already a significant increase in autoanti-
body levels at 2 mo of age. This increase was augmented at 3 mo, consistent with a proportional increase in total Ig. In correlation with the positive Coombs’ test mice developed anemia. Four WT mice and five age-matched LatY136F mice showed significant differences in hematocrit (control mice: hematocrit 50; Coombs’ test-positive mice, hematocrit 30).

At 2 as well as 3 mo of age, we observed signs of severe glo-

merulonephritis and up to 60% of mice displayed proteinuria at the later time point (Fig. 6E). In all mice with albuminuria, over 1 mg/ml as determined by ELISA was found. Albuminuria in control mice was 8 ± 5 µg/ml. An increase of blood urea from <25 µg/ml in control mice to 80 µg/ml in LatY136F mice developed. A clear relationship between albuminuria, serum urea levels, and premature death was observed. In correlation with the increased inci-
dence of proteinuria and serum urea levels, mice developed sig-
nificant glomerular lesions, which showed features of slightly proliferative mesangial glomerulonephritis characterized in partic-
ular by periodic acid-Schiff-positive deposits in the mesangium, accompanied by tubular cast formation (Fig. 6A). Extracapillary proliferation was observed occasionally.

Immunofluorescence analysis revealed linear deposits of IgE along glomerular capillary walls, together with granular IgE de-
posits in mesangium of diseased glomeruli in all the mice ana-
yzed, while no detectable deposits of other Ig isotypes (IgG1, IgG2a, and IgM) were observed (Fig. 6C). In addition, a fraction of mice (two of seven) displayed significant deposits of C3 in mesangium (Fig. 6B).

In addition to renal lesions, the lungs and liver of LatY136F mice showed striking alterations. The most interesting changes were ob-
served in lungs, which showed dense bronchovascular lymphoid infiltrates consisting of small lymphoid cells, plasma cells, and scattered large immunoblasts (Fig. 7A). Strikingly, the FDC net-

works observed in the lymph nodes and spleen were also observed in these bronchial and perivascular lung infiltrates which were stained with the FDC-specific CNA.42 Ab (Fig. 7, B and C). Lymphoid infiltrates with plasma cells comparable to those found in lungs were also observed in portal spaces of the liver (Fig. 7D). We did not detect basophil or eosinophil infiltrates in the kidneys, but eosinophil-infiltrates were detected readily in the spleen. In addition to lymphoid cell infiltrates, variable numbers of basophils/mast cells and eosinophils were also found in liver and lung les-

tions. These data indicate that LatY136F mice developed chronic systemic autoimmune disease, which was apparent already at the age of 2–3 mo.

Discussion

We have characterized the B cell response induced by the poly-

clonally activated T cell population that expands in LatY136F mutant mice in the absence of any unintentional immunization and in-

vestigated the consequences for autoimmunity. We show here that naive B cells are activated polyclonally and form massive GC, as well as plasma cells and memory B cells. A new plasmablast subset expressing CD93 was identified in LatY136F mutant mice, and its presence was confirmed in antihapten or antiviral responses (data not shown). The effector Th2 bias of the proliferating CD4 T cells induced noncomplement-fixing IgG1 and IgE isotypes in the serum as previously shown (20, 21). This B cell response did not require Ag/TCR-dependent interaction between Th2 cells and B cells. The response was clearly polyclonal without any bias for autoantibodies, leading to severe Th2-mediated systemic autoim-
munity with nephritis, IgE, and C3 in the kidneys, proteinuria, and increased serum urea levels.

Polyclonal B cell activation by LatY136F T cells

In LatY136F mice, the very few double-positive thymocytes that successfully matured to CD4+ and CD8+ single-positive stages had been selected on MHC class II and class I molecules, respec-
tively (20). To explain the presence of a lymphoproliferative dis-

ease and of autoantibodies in LatY136F mutant mice, it was origi-
nally hypothesized that this mutation resulted in incomplete negative selection (21). According to this hypothesis, potentially autoreactive T cells could then migrate to peripheral lymphoid organs and activate B cells, which would be expressing self pep-
tide-MHC class II complexes to which the T cell repertoire had not been negatively selected. Using LatY136F mice expressing the HY TCR, an MHC class I-restricted TCR originally calibrated in a LAT-proficient context, it has been shown recently that the “window” for positive and negative selection has been shifted in LAT mutant mice (34). Therefore, it is likely that CD4 T cells developing in LatY136F mice are nevertheless appropriately selected in the context of the crippled LAT molecules (19). In the periphery, LatY136F mutant T cells express very low levels of surface TCR and show undetectable calcium-influx after TCR and CD28 cross-linking (34). Consistent with these observations, we showed that LatY136F mutant T cells did not require MHC class II expression by WT polyclonal B cells in order for them to induce IgG1 secretion. In addition, in LatY136F mutant mice, titers of anti-DNA, anti-laminin, anti-chromatin, anti-NP, and anti-TNP Abs were in-

creased proportionally to the increase in total Ig. Therefore, we can exclude preferential activation of autoantibody-producing B cells. The presence of autoantibodies in LatY136F mice may reflect the fact that the LatY136F CD4 effector T cells help B cells in a “quasi-

mitogenic” mode, thereby inducing a massive polyclonal B cell
activation that is accompanied by the production of Abs to multiple specificities, including autoantigens. It has been shown previously that polyclonally activated T cells can induce B cell proliferation and Ab production in naive polyclonal B cells and that this effect is not strictly dependent on expression of MHC class II molecules by the B cells (35, 36). This is highlighted by the fact that MHC class II-deficient mice do form GC after adoptive transfer of Lat136F T cells. The observation that, after adoptive transfer of Lat136F mutant T cells into MHC class II-deficient mice, GC were smaller than those in MHC class II-expressing recipients nevertheless shows that MHC class II molecules do play a role in the GC reaction. We currently have no explanation why in Lat136F mice. MHC class II interactions may be required for optimal GC formation/maintenance. So far, we were not able to detect an Ag-driven induction of the Ab response after immunization of Lat136F mice (data not shown). Alternatively, the homeostasis of B cells and T cells may be altered in MHC class II-deficient mice.

**Polyclonal B cell response and Th2 autoimmunity**

As described above, the B cell response in Lat136F mice is truly polyclonal without any obvious bias for autoantigens. Since the activating T cells are Th2 effector cells, the hyperimmunoglobulinemia is restricted to IgG1 (100-fold) and IgE (225,000-fold). Total Ig increases by a factor of 30-fold in 2- to 3-mo-old Lat136F mice. Similar observations were made for Th1 responses in lpr or gld mice (37).

The association of lupus nephritis with Th1 responses has become clear (38). Introducing a Th2 bias results in protection from lupus (39, 40). To exclude a direct effect of IL-4 on nephritis, we crossed Lat136F mice with CD40 knockout mice abolishing lupus susceptibility despite strong Th2 accumulation. These double-knockout mice show delayed increase in IgG1 and IgE Ab titers (our unpublished observations).

**Autoimmune nephritis**

Most classical human and mouse autoimmune diseases, including lupus, are clearly multigenic (1, 2). Increased total Ig has been associated frequently with lupus, leading to immune deposits in renal glomeruli. In the classical murine models of lupus, (NZB × NZW)F1, MRL-Fas8/w, and BXSB, autoantigen-driven expansion of autoreactive plasma cells is observed, and the resultant Abs show signs of affinity maturation with variable increases in affinity for abundant autoantigens such as chromatin and DNA (for review, see Ref. 41). Alternatively, polyclonal B cell activation models leading to nephritis have been described previously, B cell-activating factor of the TNF family (BAFF) transgenic mice, which display polyclonal hyperimmunoglobulinemia and systemic autoimmunity with lupus-like features, are the most similar to Lat136F mice (6). However, in our mice, no increase in serum BAFF levels was observed (data not shown). In classical murine lupus, however, pathogenic IgG2a Abs, which efficiently activate complement and interacts with both FcγRI and FcγRIII, have been implicated in the disease. A recently described new FcγRI, FcγRIV, which does not fix IgG1 isotypes, might further explain the higher disease potential of IgG2a Abs (42). In Lat136F mutant mice, the Th2-induced, noncomplement-fixing IgE Abs form IgE deposits in the local tissue, lung infiltrates (×50 and ×200).

**FIGURE 6.** Glomerulonephritis developed by Lat136F mice. A, H&E staining of kidney sections of littermate or 3-mo-old Lat136F mice. B, Staining of kidney sections with anti-C3 to detect complement deposits. Top panels, WT; bottom panels, Lat136F; left panels, small enlargement (×10); and right panels, larger magnification (×40). C, Analysis of Ig deposits by fluorescence staining with anti-IgG1 and anti-IgE (left panels, anti-IgG1, ×10; middle panels, anti-IgE (small enlargement, ×10); and right panels, anti-IgE (large magnification, ×40). D, Quantification of kidney lesion. E, Proportion of Lat136F mice positive for proteinuria. Significant differences as calculated by the Mann-Whitney rank-sum test for disease index and by Fisher’s exact test for proteinuria analysis are indicated. *, p < 0.05; and **, p < 0.01.

**FIGURE 7.** Lung and liver pathology of Lat136F mice. A, H&E staining of Lat136F lung showing peribronchial and perivascular lymphoid infiltrates (×50). B and C, Low and high power magnification of FDC staining in lung infiltrates (×50 and ×200). D, lymphoid infiltrates in the portal space of the liver at ×200.
the glomeruli and induce early-onset nephritis. Immunohistology supports a predominant role for IgE in glomerular injury in association with complement deposition in two of seven mice.

Importantly, for our study, several models exist where single recessive mutations result in spontaneously activated T cells and the subsequent production of autoantibodies. Two examples are the Zap mutation or the use of autoreactive TCR transgenic mice (43, 44). Forward genetics has revealed a mutation in a RING-type ubiquitin ligase that increases the activity of follicular Th cells, resulting in autoimmune nephritis (45) via abnormally strong help (43, 44). Forward genetics has revealed a mutation in a RING-type ubiquitin ligase (LatY136F) in man and in mouse (50–55). These features are observed in lupus-prone mice spontaneously developing perivascular lymphoid hyperplasia with plasmocytes and lymphocytes (49). Perivascular infiltrates of liver are a recurrent feature of lupus in man and in mouse (50–55). These features are observed in LatY136F mutant mice. In addition, FDC are readily detectable in the perivascular lymphocyte infiltrates in the lung. Such neofor- mation of GC-like structures is seen repeatedly in rheumatoid arthritis and Sjogren syndrome (56, 57).

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Currently, we have neither proof nor an explanation for a pathogenic role of IgE in the kidneys of LatY136F mice. Several Th2-mediated autoimmune diseases with IgE deposits have been described previously. Interestingly, IgE deposits have been described in human diseases. Increased IgE levels were detected in some lupus patients (58), and IgE deposits found in ~10% of cases in human renal disease were correlated with bad prognosis (59–63). Similarly, diseases related to parasitic infections might involve IgE immune deposits (64, 65). HgCl2-induced allergic reactions have been shown in patients with severe allergic asthma (66). In addition, there is a role for IgE deposits in Wegener’s granulomatosis, as well as IgE myelomas (67, 68). For most of these diseases, a direct role of IgE deposits in Wegener’s granulomatosis has been suggested but not yet been shown conclusively.


