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Megakaryocyte Progenitors Are the Main APCs Inducing Th17 Response to Lupus Autoantigens and Foreign Antigens

Hee-Kap Kang,* Ming-Yi Chiang,* Diane Ecklund,* Li Zhang,* Rosalind Ramsey-Goldman,* and Syamal K. Datta*†

In search of autoantigen-presenting cells that prime the pathogenic autoantibody-inducing Th cells of lupus, we found that CD41+ CD151+ cells among Lineage– (Lin–) CD117+ (c-Kit+) CX3CR1+ splenocytes depleted of known APCs were most proficient in presenting nuclear autoantigens from apoptotic cells to induce selectively an autoimmune Th17 response in different lupus-prone mouse strains. The new APCs have properties resembling megakaryocyte and/or bipotent megakaryocyte/erythroid progenitors of bone marrow, hence they are referred to as MM cells in this study. The MM cells produce requisite cytokines, but they require contact for optimal Th17 induction upon nucleosome feeding, and can induce Th17 only before undergoing differentiation to become c-Kit+ CD41+ cells. The MM cells expand up to 10-fold in peripheral blood of lupus patients and 49-fold in spleens of lupus mice preceding disease activity; they accelerate lupus in vivo and break tolerance in normal mice, inducing autoimmune Th17 cells. MM cells also cause Th17 skewing to foreign Ag in normal mice without Th17-polarizing culture conditions. Several molecules in MM cells are targets for blocking of autoimmunization. This study advances our understanding of lupus pathogenesis and Th17 differentiation biology by characterizing a novel category of APC.


Systemic lupus erythematosus (SLE, or lupus) is a major systemic autoimmune disease with complex genetically determined effects on the immune system. MHC class II genes are major determinants of lupus susceptibility, underscoring the importance of autoantigen presentation by class II molecules to autoreactive Th cells in initiating the disease (1). In lupus autoimmunity, pathogenic IgG autoantibodies that fix complement and bind FcγR on inflammatory cells are produced with help from Th1 and Th17 cells that are specific for peptides from nucleosomes or ribonucleoproteins of apoptotic cells, and such Th cells also infiltrate vital organs (2–11). Macrophages (e.g., tingible body macrophage) and dendritic cells (DCs) are normally tolerant to apoptotic cell Ags (12), but they are activated to present such autoantigens after binding by FcγR to IgG immune complexes containing apoptotic cell-derived DNA/RNA, which then dually stimulate via their TLR and FcγR (13–18). Hence, to generate the activating IC, IgG-switched autoantibodies have to be made first by T cell help. Moreover, B cells become efficient APCs to Th cells preprimed by other APCs (19), and B cells can be stimulated by nuclear Ags synergistically via BCR and TLR after developing high-affinity somatically hypermutated receptors with T cell help (20, 21), otherwise anti-DNA B cells are inactivated (22, 23). Thus, conventional APCs are essential for disease progression, but it is unknown what initially primes autoimmune Th cells. We fractionated spleen cells of lupus-prone mice in search of such an APC.

Materials and Methods

Mice

NZB and SWR mice were purchased from The Jackson Laboratory (Bar Harbor, ME) to breed lupus-prone SNF1 (SWR × NZB)F1 hybrids (24). Female SNF1 mice, like BWF1, have high serum levels of IgG class anti-DNA and other anti-nuclear autoantibodies by 2 mo and spontaneously begin to develop severe lupus nephritis by 5 mo of age (25). The other lupus-prone recombinant congenic strain B6.Sle (BcN/LmoJ or B6.Sle1,2,3) homozygous for the NZM2410 lupus susceptibility QTL (Sle1, Sle2, Sle3) on the normal C57BL/6J background (26), normal C57BL/6 (B6), and OTHI transgenic [B6.Cg-Tg(TeraTcrb)425Cbn/J] with T cells specific for OVA peptide OVA123–139 on 1-Aβ were from The Jackson Laboratory. Females were used, and all studies were approved by the institutional animal care and use committee.

Human subjects

Ten patients in clinical remission (all females; aged 27–63 y) fulfilling American College of Rheumatology revised criteria for SLE (27) and six normal (healthy) subjects (five females and one male; aged 22–46 y) were studied with approval by the institutional review board. Disease activity by Systemic Lupus Activity Measure (SLAM) (5, 28, 29) ranged between 1 and 8 or that by Systemic Lupus Disease Activity Index (SLEDAI) ranged between 0 and 2 (30) for the patients. Demographic data for the lupus patients including clinical and treatment status are shown in Table I.

Cell isolation

CD90+, CD4+, CD8+, CD19+, γδ+, DX5+, CD117+ (c-Kit+), CD11c+, CD11c+, Gr-1+, and FcεRI+ cells from spleens were purified by magnetic bead conjugated Abs (Miltenyi Biotec, Auburn, CA). Lin–c-Kit+ pure cells were isolated using anti-CD117 beads after depletion of the above markers.
twice. Mast cells were isolated by anti-FcεRI–FITC and anti-FITC beads. CD4 CD8 T cells were purified as before (10, 31). Cell subsets were >90% pure.

**ELISPOT assay**

ELISPOT plates were coated with capture Abs to IFN-γ or IL-17 (BD Biosciences, San Diego, CA) as published (8, 32). On the basis of optimal cell ratios established from dose-response curves, T cells (1 × 10^5) were cultured with APC subsets (0.25 × 10^6 each) from 5-mo-old SNF1 mice in the presence of nucleosomes or saline. Cytokine-expressing cells were detected at 24 h for IFN-γ or 48 h for IL-17. Lin−c-Kit+ cells cultured by themselves without nucleosomes or with nucleosomes (10–30 μg/ml) produced 30 ± 5 and 34 ± 6 spots, respectively. T cells plus Lin−c-Kit+ cells in coculture without nucleosomes produced 15 ± 8 spots (background), and so did T cells (1 × 10^5) cocultured with low numbers (0.05 × 10^6) of Lin−c-Kit+ cells even in the presence of nucleosomes. Th17 response in OTII transgenic naive T cells to OVA peptide OVA323–339 molecules in responding T cells were measured with primers as described (30, 31, 32)

**Blocking of Th17 response to nucleosomes by anti-MHC class II Abs**

T cells (1 × 10^5) were cultured with APC subsets (0.25 × 10^6 each) from 5-mo-old SNF1 mice in the presence of anti-MHC class II Abs (clone M5/114; eBioscience, San Diego, CA) or isotype control stimulation with stimulation with nucleosomes in IL-17 ELISPOT plates. Anti-MHC class II Abs were preincubated with APCs for 1 h before addition of nucleosomes and T cells. Blocking of Th17 response to nucleosomes by anti-MHC class II Abs was analyzed by comparing isotype control in the 48-h ELISPOT assay.

**Autoantibody quantitation**

IgG class autoantibodies to ssDNA, dsDNA, and histone and nucleosome (histone–DNA complex) were measured by ELISA (3, 4).

**Helper assay for IgG autoantibody production in vitro/ex vivo**

To detect autoantibody-inducing help, CD90+ T (1 × 10^5/well) or purified CD4+ T (0.25 × 10^5/ml) cells from 5-mo-old SNF1 mice in culture for 7 d and then supernatant analyzed for IgG autoantibodies.

**Phagocytosis**

Engulfment of noncarboxylated fluoresceinated latex beads (2.1 μm; Polyscience Warrington, PA) or CFSE (Invitrogen, Carlsbad, CA)–labeled and irradiated apoptotic thymocytes at various ratios, at 37˚C for 6–72 h, by Lin−c-Kit+ or other APC was assessed by staining with allophycocyanin–anti-CD11b or PE–anti-CD11b before incubation with latex beads or apoptotic thymocytes for microscopy, but stained after the incubations for flow cytometry. Apoptotic thymocytes were prepared by gamma-ray irradiation (3000 rad) and stained with annexin V and propidium iodide after 6–12 h to confirm apoptosis.

**DQ-OVA endocytosis**

BODIPY-conjugated DQ-OVA (D-12053; Molecular Probes, Eugene, OR) exhibits bright-green fluorescence upon proteolytic processing. DQ-OVA (10 μg/ml) was added to Lin−c-Kit+ cells (5 × 10^5 cells) and incubated at 4˚C or 37˚C for 20 min to 2 h. Controls include 4˚C with DQ-OVA or without DQ-OVA and 37˚C without DQ-OVA, according to the manufacturer’s references. Cells were then washed and analyzed by flow cytometry.

**Flow cytometry**

Cells were stained with allophycocyanin–conjugated anti-CD117, FITC– or PE–anti-CD11b, FITC– or PE–anti-CD11c (BD Pharmingen), PE– or allophycocyanin–anti-CD41 and PerCP/Cy5.5–anti–Gr–1 (eBioscience), PE–anti-CD151 (R&D Systems), PE– or PerCP/Cy5.5–anti-CD150, Pacific blue–DQ48 (BioLegend, San Diego, CA), or matched isotype controls at 4˚C for 30 min, then acquired by LSR II with FACS Diva (Becton-Dickinson) at our Immunobiology Center core facility and analyzed using FlowJo (Tree Star, Ashland, OR). For sorting, c-Kit+ cells were isolated from Lin− splenocytes by magnetic beads and then stained with FITC– or PerCP/Cy5.5–anti-CD150, PE–anti-CD151, allophycocyanin–anti-CD105, PE–Cy7–anti–Sca–1, and Pacific blue–anti-CD44 at 4˚C for 30 min and sorted by MoFlo (Dako Cytomation, Carpinteria, CA) with Summit and FACS express 3 software (De Novo Software, Los Angeles, CA) at our Robert H. Lurie Comprehensive Cancer Center core facility. For identification of MM cells in human peripheral blood mononuclear cells (PBMCs), PBMCs were prepared by Ficoll-Paque (GE batches were Biosciences, Piscataway, NJ) gradient of whole blood from lupus patients anduffy coats or whole blood from healthy donors. PBMCs were stained with Alexa Fluor 700–anti-CD3, eFluor anti-CD19, ECD–anti-CD14, allophycocyanin–Cy7–anti-CD16, allophy-
coercytin-anti-CD117, FITC-anti-CD41, and PE-anti-CD151 Abs. After gating for CD3+ CD19+ CD14+ CD16+ fraction of cells in PBMCs, CD117+HpheCD14+ cells were gatted from that fraction first, and all of them were CD151+. CD151Hphe+ cells among CD117+HpheCD41+ cells were then gatted.

Hematopoietic lineage potential

Sorted Lin–c-Kit+ cell subsets were cultured in MethoCult M3134 with 2% FBS for CFU or IMDM with 10% FBS with mouse stem cell factor (SCF; 50 ng/ml), IL-3 (20 ng/ml), thrombopoietin (TPO; 40 ng/ml), and EPO (20 ng/ml) (R&D Systems; Stemcell Technology), and then stained for CD42b and Ter 119 for megakaryocytes and erythrocytes by FACS and Giemsa (37).

Cell cloning by limiting dilution

Lin–c-Kit+ cells were cultured at a density of 100, 50, 10, 2, or 0.5 cell in RPMI 1640 with 10% FBS with SCF (50 ng/ml). Culture supernatants of Lin–c-Kit+ cells were used with 1:1 fresh medium for expanding clones.

Statistical analysis

Chi-square test, log rank test, and the Student two-tailed t test were used. Results are expressed as mean ± SEM.

Results

Lin c-Kit+ pure cells almost exclusively induce Th17 responses to nuclear autoantigens without undergoing further differentiation

We depleted spleen cells of lupus-prone SNF1 mice of cells with mature lineage markers, including conventional APC (CD90+, CD3ε+, CD19+, B220+, CD11c+, CD11b+, CX3CR1+, NK1.1+, Gr-1+, Ter119+, and FceRI+ mast cells-depleted) and then isolated pure CD117+ (c-Kit+, or K+) cells from the lineage− (Lin−, or L−) cells (Supplemental Fig. 1A). These Lin–c-Kit+ pure cells, which had the morphology of hematopoietic progenitor cells, were almost the sole inducers of nuclear autoantigen-specific Th17 responses (Fig. 1A–C). CD11c+ DCs isolated before pulling out CD11b+ and CD117+ cells, thus including few CD11c+CD117low+ cells, induced mainly nucleosome-specific Th1 response, but very low Th17 (p = 0.003 to 0.00004 versus Lin–c-Kit+ pure cells). The Lin–c-Kit+ pure cells presented autoantigens without differentiation into DCs or macrophages because they could process/present nucleosomes to induce a Th17 response even after irradiation (3000 rad) or culturing and cloning in media supplemented with SCF (c-KitL) alone that prevented further differentiation (Fig. 1D–G). The ELISPOT assays to measure T cell responses in all of the above and subsequent experiments included background controls with APCs alone, T cells alone, and APCs plus T cells without nucleosomes. The Lin–c-Kit+ pure cells cultured by themselves without nucleosomes or with nucleosomes (at 10–30 μg/ml) produced 30 ± 5 and 34 ± 6 spots, respectively, at background levels (representative examples shown later in Figs. 3A, 6C); and coculture of these APCs with T cells without nucleosomes produced only a few background spots (15 ± 8), as stated also in the figure legends and in Materials and Methods. Moreover, nucleosome-activated Lin–c-Kit+ pure cells did not express IL-17 message by quantitative PCR or microarray (Supplemental Table I).

Lin c-Kit+ pure cells express requisite cytokines but are also contact dependent for optimal Th17 induction

Freshly isolated or SCF-cultured Lin–c-Kit+ pure cells from spleens of SNF1 mice expressed mRNAs of Th17-inducing cytokines (38–41) upon incubation with LPS or nucleosomes, which respectively increased IL-6 mRNA by 27- and 10-fold, TGF-β mRNA by 108- and 18-fold, TNF-α by 38-fold for both, IL-18 by 9- and 5-fold, and IL-1β mRNA by 126- and 90-fold at the 6-h time point, but the levels of IL-23/IL-12 (p40) and TL1a (43) mRNAs did not increase (Fig. 2A, 2B, and data not shown). Therefore, Lin–c-Kit+ pure cells might also express other mediators facilitating expansion of precocommitted Th17 cells in...
addition to Th17 induction and priming (described later). Nucleosomes did not stimulate the Lin-c-Kit\textsuperscript{+}pure cells due to any LPS contaminant, as addition of polymyxin B or anti-TLR4 Ab did not inhibit Th17 response elicited by nucleosome-pulsed Lin-c-Kit\textsuperscript{+}pure APCs, and no differences in endotoxin levels were found between nucleosome preparation and PBS or culture media using an endotoxin detection kit (data not shown). Notably, Transwell cultures showed freshly isolated Lin-c-Kit\textsuperscript{+}pure cells are contact dependent for inducing nucleosome-specific Th17 response (Fig. 2C), indicating that these APCs induce Th17 by secreted and surface molecules working at close range.

**Lin-c-Kit\textsuperscript{+}pure cells have APC machinery to efficiently process and present apoptotic cells and nuclear autoantigen particles**

Like other APCs, Lin-c-Kit\textsuperscript{+}pure cells expressed MHC class II and costimulatory molecule CD86, and with nucleosomes or TLR ligands LPS and CpG increased MHC class II on their surface. Anti-MHC class II Abs blocked Lin-c-Kit\textsuperscript{+}pure cell-induced Th17 responses significantly. The Lin-c-Kit\textsuperscript{+}pure cells phagocytosed latex beads and apoptotic cells, and they also could process foreign Ag OVA comparable to DCs, and microarray showed they were 27-fold (p = 0.000002) and 9-fold (p = 0.009) by LPS and 10-fold (p = 0.00023) and 5-fold (p = 0.0015) by nucleosome pulse, respectively. (G) Lin-c-Kit\textsuperscript{+}pure cells fed with nucleosomes were separated from cocultured T cells by Transwell membranes in IL-17 ELISPOT plates, and the responses of the T cells are shown. (D and E) Lin-c-Kit\textsuperscript{+}pure cells express MHC class II and CD86 (D), and their ability to induce Th17 response to nucleosomes was blocked by anti-MHC class II Abs (E). (F) Lin-c-Kit\textsuperscript{+}pure cells efficiently process OVA (DQ-OVA). (G) Representative histograms of CFSE-labeled apoptotic cells engulfed (engulfment was confirmed by microscopy, see Materials and Methods; similar to Supplemental Fig. 2, also see Fig. 4B). ▼ indicates apoptotic cell engulfed by CD11b\textsuperscript{+} (left panel) or Lin-c-Kit\textsuperscript{+}pure cells (right panel). (H) Percent of apoptotic cell engulfed by cell subsets in FACS analysis. Mean ± SEM of five separate experiments.

**CX3CR1\textsuperscript{+}c-Kit\textsuperscript{+} cells differentially express genes related to megakaryocyte progenitors**

CD117 (c-Kit) is also expressed by macrophage/dendritic cell precursors (MDP), which also express the chemokine receptor CX3CR1 (47–49). Therefore, from T and B cell depleted spleen cells, we sorted out CD117\textsuperscript{CX3CR1}, CD117\textsuperscript{CX3CR1\*} (MDP), CD117\textsuperscript{CX3CR1\*}, and CD117\textsuperscript{CX3CR1} cells, and then tested their abilities to induce Th1 and Th17 responses to nucleosomes. CD117\textsuperscript{CX3CR1\*} cells were Lin\textsuperscript{−} (Lin-c-Kit\textsuperscript{−}CX3CR1\textsuperscript{−}, or L’K\textsuperscript{−}C\textsuperscript{−}), and they induced solely Th17 response, whereas CD117\textsuperscript{CX3CR1\*} cells induced only Th1 response, but CD117\textsuperscript{CX3CR1\*} (L’K\textsuperscript{−}C\textsuperscript{+}) cells induced high Th1 and low Th17 responses (Fig. 1), which are also CX3CR1\textsuperscript{−}, although isolated in a different way. Therefore, we compared gene expression profiles of nucleosome-pulsed, Th17-inducing APCs that were isolated in...
FIGURE 3. Linc-Kit\textsuperscript{pure} cells induce Th17 response by different subsets of T cells and induce molecules expressed by Th17 cells. (A) Lin c-Kit\textsuperscript{pure} cells fed with nucleosomes (10 \( \mu \)g/ml) were cocultured as APCs with \( \gamma^8 \) T cells, CD4\textsuperscript{T} cells, or CD4\textsuperscript{CD8} T cell subsets fractionated from SNF1 splenocytes, and the responses of the T cells in IL-17 ELISPOT plates are shown, in addition to the IL-17 spots produced by Lin c-Kit\textsuperscript{pure} APCs cultured by themselves in presence of nucleosomes (APC alone). The cells were isolated from 5-mo-old female SNF1 mice. T cells and Lin c-Kit\textsuperscript{pure} cells cultured without nucleosomes produced 13 \pm 6 spots as background. (B) Lin c-Kit\textsuperscript{pure} cells and T cells from SNF1 mice were cocultured in the presence of 20 \( \mu \)g/ml nucleosomes and then analyzed for relative expression of mRNA of ROR\textgamma (ROR\gamma), integrin \( \beta_1 \) (Ig\textsubscript{b1}), integrin \( \beta_4 \) (Ig\textsubscript{b4}), IL-23R, and IL-21 by real-time PCR at various time points. T cells alone or Lin c-Kit\textsuperscript{pure} cells alone did not express these genes. Mean \pm SEM of three experiments are shown, \( n = 15 \) mice.

Those two ways, namely purified Lin c-Kit\textsuperscript{pure} (L\textsuperscript{K}\textsuperscript{pure}) cells or the Lin c-Kit\textsuperscript{CX3CR1\textsuperscript{+}} (L\textsuperscript{K}\textsuperscript{CX\textsuperscript{+}}) cell subset, with Th1-inducing APCs, namely Lin c-Kit\textsuperscript{CX3CR1\textsuperscript{+}} (L\textsuperscript{K}\textsuperscript{CX\textsuperscript{+}}) subset, or CD11c\textsuperscript{+}CD11b\textsuperscript{low}c-Kit\textsuperscript{cells} (DCs). We purified CD11c\textsuperscript{+}CD117\textsuperscript{cells} from total DCs to compare (and contrast) with the former two cell preparations because c-Kit\textsuperscript{+} (CD117\textsuperscript{+}) DCs that are present in total CD11c\textsuperscript{+} cells can induce nuclear autoantigen-specific Th17 cells, albeit to a much lower extent than the former (Fig. 1A, 1B, Supplemental Fig. 1B–E). Spleen cells from three batches of three 5-mo-old SNF1 female mice per batch were used to perform three independent isolations of each cell sub-population. By analyzing the functions of 230 genes that were significantly and differentially upregulated in all four pair comparisons (see Materials and Methods and microarray data in Supplemental Table I), the two Th17-inducing APC isolates, namely Lin c-Kit\textsuperscript{CX3CR1\textsuperscript{+}} (L\textsuperscript{K}\textsuperscript{CX\textsuperscript{+}}) cell subset or purified Lin c-Kit\textsuperscript{pure} (L\textsuperscript{K}\textsuperscript{pure}) cells, highly expressed genes for transcription factors Gfi-1B, GATA1, GATA2, NF-E2, Meis1, and...
with highly significant changes (3- to 22-fold), we further fractionated Lin–c-Kit+CX3CR1– cells by sorting them to CD41+ cells, hematopoietic stem cell (HSC), multipotent progenitor 1 (MPP1), multipotent progenitor 2 (MPP2), and CFU-erythroid progenitor (CFU-E) (Fig. 6A and Supplemental Fig. 3A, Supplemental Table I). In contrast, the c-Kit+CD41+ cells in the right lower quadrant of Fig. 7A, did not express CD151 in the mice or induce Th17 response to nucleosomes (11 ± 4 spots), and those cells would be included in all Lin–c-Kit– APC preparations, none of which were able to induce a Th17 response (Figs. 1, 5, 7). The MM cells in lupus-prone SNF1 spleens were increased up to 49-fold more (p = 0.00000001) than in normal C57BL/6 (B6), increasing with age, but the SNF1 MM cells’ Th17-inducing ability was similar irrespective of age (Fig. 7B and data not shown). Similar to nucleosomes (8), whole apoptotic cells also stimulated Th17 response in splenocytes of lupus-prone SNF1 female mice (p = 0.0387 to 0.000136), but not in normal SWR, which have very few MM cells (data not shown). Another lupus-prone strain, B6. Slr, also had up to 46-fold increase of MM cells (p = 0.00000021) compared with that of B6. Even though measurement was feasible only in peripheral blood (not spleens), lupus patients (in clinical remission) also showed 4.8-fold increase in MM cells (p = 0.0000025) and 10.4-fold increase in CD151high MM cells (p = 0.00026) gated for CD117HighCD41+ cells in peripheral blood compared with normal humans (Fig. 7E). There was no correlation between frequency of MM cells and the clinical activity scores (SLEDAI or SLAM) in these patients (Table I).

**MM cells break tolerance in normal mice and induce Th17 skewing in response to foreign Ags without polarizing culture conditions**

The MM cells from B6.Sle induced Th17-skewed responses to nucleosomes like SNF1, and remarkably they could induce Th17 differentiation in naïve, normal B6 (Fig. 7F) without the polarizing culture conditions required by other APC types (54). However, cells of the MM phenotype purified from large batches of B6 spleens lacked the ability to induce autoimmune Th17 (Fig. 7F). Remarkably, MM cells purified from B6.Sle mice could also elicit a Th17-dominant response to foreign Ag, OVA (OVA peptide323–339), in the T cells bearing receptors for OVA peptide323–339 of transgenic mice of normal B6 background without artificial polarizing culture conditions or PMA–ionomycin stimulation. In contrast, splenic APCs of the OTII mice themselves failed to induce Th17 (Fig. 7G). Thus, MM cells are Th17-inducing APCs in general.

**Molecular targets in MM cells for blocking autoimmune response**

Lin–c-Kit+CX3CR1+ cells expressed megakaryocyte/platelet-related molecules (Fig. 5D, Supplemental Table I). By quantitative PCR and flow cytometry of differentially expressed gene products, we validated and narrowed down candidate molecules in MM cells, which could be tested to inhibit the c-Kit+ MM cells themselves from presenting autoantigens or producing known Th17-inducing cytokines and/or interfere with close-range (contact-dependent) interactions necessary for inducing Th17 cells, after nucleosome pulsing. We also tested whether blocking of the selected target molecule on MM cells pulsed with nucleosomes can suppress their ability to stimulate T cell helper function...
for B cells to produce IgG autoantibodies in coculture using established methods (33, 34, 35). Among all those candidates tested (Fig. 5D), positive results are described in this study. Ab to the tetraspanin CD151 blocked nucleosome-stimulated anti-DNA autoantibody production and induction of Th17 response by Lin−c-Kit+CX3CR1+ APC, but not induction of Th1 response by Lin−c-Kit+CX3CR1+ APC subset (Fig. 8A, 8B); the former APC subset predominantly induces Th17, whereas the latter induces Th1 response to nucleosomes (Fig. 5B, 5C). CD151 could stabilize interaction of MM cells by binding to integrin αIIbβ3 and β4 on Th17 cells (56, 57), whose expression was markedly augmented in the T cells when cocultured with MM cells pulsed with nucleosomes (Fig. 3B), and anti-CD151 did not inhibit MM cells directly (Fig. 8C). CD151 is also expressed by other cells and APCs (Supplemental Fig. 3B, Supplemental Table I). We also obtained positive results by targeting CD41, the MM surface molecule (Fig. 5D, Supplemental Table I). CD41 is an integrin subunit expressed on MM cell membranes and hypothetically could augment Th17 induction by increasing adhesive interactions and cell surface signaling. The CD41 integrin subunit, αIIbβ3, forms a noncovalently linked complex with β1 to be expressed on the cell surface (http://www.ncbi.nlm.nih.gov/gene?term=3674) and functions as a receptor for fibronectin and VWF. However, β3 integrin subunit message was not differentially increased in MM cells subunit message as a receptor for fibronectin and VWF. However, CD151 could augment Th17 induction by increasing adhesive interactions and cell surface signaling. The CD41 integrin subunit, αIIbβ3, forms a noncovalently linked complex with β1 to be expressed on the cell surface (http://www.ncbi.nlm.nih.gov/gene?term=3674) and functions as a receptor for fibronectin and VWF. However, β3 integrin subunit message was not differentially increased in MM cells.

**Discussion**

The series of experiments in this study show that among Lin−c-Kit+ cells depleted of conventional APCs and mast cells, the most efficient presentation of apoptotic cell nuclear Ags for inducing autoimmune Th17 cells is a property of the CD41+CD151+ subset of c-Kit+CX3CR1+ cells, which could be made to differentiate into megakaryocyte/platelet and erythrocytes with appropriate growth factors (similar to Mkp and MEP in BM), but they lost autoantigen-presenting and Th17-inducing ability upon differentiation to c-Kit+CD41+ cells. Thus we refer to this new type of APC resembling bone marrow Mkp and MEP progenitor cells as MM cells because they are found in the lupus spleen with nuclear autoantigen-presenting function, express previously unreported marker molecules in addition to possessing Ag processing machinery, and are capable of producing requisite Th17-inducing cytokines and costimulators. The MM cells lack receptors for...
FLT3 ligand and M-CSF (data not shown), besides lacking CX3CR1; thus they do not have myeloid or DC–macrophage progenitor markers (47–49). Moreover, the earliest progenitor-like cells in the lupus splenic Lin–c-Kit+ cells that resemble HSC and MPP1 and MPP2 cells of BM (52, 53) did not have Th17-inducing capability. Another variety of Lin–c-Kit+ APCs, totally different from MM cells in this study, are generated by deliberate stimulation with IL-25; they are multipotent progenitor-like Th2-inducing cells residing in GALT, and they differentiate into macrophage and granulocyte lineages (60). Th17-inducing DC subsets have been detected in other systems (61, 62), but c-Kit stimulation of the DC or PMA and ionomycin stimulation of the T cells was required to detect Th17 response in those cases in contrast to the Th17-inducing MM cells of this study. Moreover, the Th17-inducing ability of c-Kit+ DCs was miniscule compared with that of MM cells in this study under natural autoantigen-presenting conditions (Fig. 1).

Notably, MM cells express megakaryocyte progenitor molecules that are also expressed by platelets (Fig. 5D, Supplemental Table I). Platelets have been implicated in lupus pathogenesis by expressing CD40L, producing cytokines, and participating in immune complex-mediated inflammation (63, 64). However, the c-Kit+ MM cells from lupus subjects isolated by flow cytometry sorting or magnetic beads had the size and morphologic characteristic of nucleated mononuclear cells (Fig. 1, Supplemental Fig. 1), which would exclude or gate out platelets, and we found in this study that only the MM cells could uptake and present nuclear autoantigens and induce Th17 cells in a class II-dependent manner before undergoing further differentiation (Figs. 1, 2). In a related

Table I. Lupus patient demographics: clinical and treatment status of SLE patients who provided samples for this study

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<td>HCQ</td>
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<td>9</td>
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<td>10</td>
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AZA, Azathioprine (Imuran); HCQ, hydroxychloroquine (Plaquenil); LEF, leflunomide (Arava); MMF, mycophenolate mofetil; Pred., prednisone or steroids; SSZ, sulfasalazine; Vit D, vitamin D.
issue, anti-CD41 injections that caused a significant decrease of nucleosome-stimulated IgG autoantibody production ex vivo by the splenocytes of the treated mice (Fig. 8D) would have also depleted platelets (58, 59), which could have helped autoimmune B cells by increased expression of CD40L. However, in lupus, both autoimmune Th and B cells hyperexpress CD40L because of intrinsic defects anyway (29, 65), and the cells from the treated animals in this study were tested for autoantibody production ex vivo in a 7-d assay.

Remarkably, Th17 induction and expansion by nucleosome-pulsed MM cells occurred without any Th17-polarizing culture conditions or PMA–ionomycin stimulation, which are generally used to detect Th17 cells in other systems. Moreover, contact was required for optimal autoimmune Th17 induction under these natural conditions indicating that MM cells, upon apoptotic cell or nucleosome feeding, require accessory molecules, such as CD151 working at close range for Th17 induction, in addition to producing known cytokines (IL-1, TGF-β, IL-6, TNF, as shown in Fig. 2A, 2B). The MM cells not only primed but also induced expansion of precommitted nucleosome-specific Th17 cells, which are increasingly seeded into the periphery of lupus mice probably due to inefficient deletion and autoreactive selection in the thymus (56, 66, 67).

Among the array of molecules expressed by MM cells (Supplemental Table I), we found that anti-CD151 blocked Th17 induction and autoantibody production (Fig. 8A–C). However, anti-CD151 did not inhibit the MM cells themselves (Fig. 8C). CD151 could facilitate interaction of MM cells with Th17 cells by increasing adhesion. Indeed, Th17 cells express the CD151 binding integrin α4β1 (56), whose expression was markedly augmented in the T cells when cocultured with MM cells pulsed with nucleosomes (Fig. 3B). Of course, CD151 or the other molecules on MM cells that could be targeted to inhibit Th17 induction, such as CD41, Pf4, and c-Kit (Fig. 8), are not uniquely specific for MM cells. Nevertheless, a combination of cytokines and surface molecules expressed by the MM cells make them potent Th17 inducers. Studies with the new type of Th17-inducing APC reveal new mechanisms for agents being beneficial in autoimmunity.

We have clearly shown that removal of Lin-ε-Kit+CD41+CD151+ cells completely abrogates Th17-inducing ability (Fig. 6C) and conversely that adoptive transfer of those cells fed with apoptotic nuclear Ags accelerate lupus disease (Fig. 4D). The preferential increase in IgG3 autoantibodies by adoptive transfer of nucleosome-pulsed MM cells was striking, although pathogenic IgG2a and IgG2b autoantibody subclasses were also increased (Fig. 4E, 4F). IgG3 autoantibodies are especially pathogenic in lupus nephritis by forming cryoglobulin immune complex deposits (68, 69). Marginal zone B cells could also be involved in producing IgG3 anti-DNA autoantibodies in lupus.
A new type of APC.

They do have smoldering disease and harbor autoimmune Th cells (31, 45), which are induced by the MM cells (Fig. 3A).

The expansion of MM cells preceding lupus disease activity and contraction of inflammatory Th17 cells are not influenced by TLR9 or other MyD88-dependent TLRs. *J. Immunol.* 179: 6663–6672.


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B cells enter the follicles and interact with CD4+ T cells in lupus-prone mice.