Introduction and Significance: The exact pathogenesis of arthritis in children or adults is not known, but several cells contribute to the development of autoimmune arthritis including T and B lymphocytes, synovial cells, macrophages, neutrophils and osteoclasts (OC). Identifying common signaling molecules affecting the osteo-immune system and their impact on normal and pathological bone loss may lay the groundwork for future therapies against inflammatory arthritis. We have found PLCγ2 to be such candidate. PLCγ2 modulates neutrophil functions, namely adhesion and cytokine release. We have also shown that PLCγ2−/− mice have an osteopetrotic phenotype due to reduced number of OCs in vivo. Thus, based on these findings and on the established role of PLCγ2 in B cell development, we hypothesized that PLCγ2 might be central for the inflammatory and osteolytic responses associated with rheumatoid arthritis.

Methods: To study the role of PLCγ2 in inflammatory arthritis we used the methylated BSA model of arthritis which is dependent on T cell activation. Mice were immunized on day 0 and day 7 by intradermal injection at the base of the tail of 100 μg mBSA (Sigma-Aldrich) emulsified in 0.1 ml Freud’s adjuvant. At the same time 1 μg pertussis toxin (List Biological Laboratories Inc.) was also injected intraperitoneally. Arthritis was induced on day 21 by intraarticular injection of 100 μg of mBSA in 10 μl PBS into the right knee, while the contralateral knee was injected with PBS alone as a negative control. Mice were sacrificed on day 32 and insurgence of arthritis was assessed by histological examination of knee sections by H&E and TRAP staining. In some experiments, purified B cells were injected by tail vein injection into PLCγ2−/− mice and presence of circulating, mature B cells was examined by FACS analysis before induction of arthritis. T cell activation was assessed by measuring TNFα/TNFβ cytokine release at the peak of the in vivo experiments. Briefly mice were sacrificed and draining ilinalymph nodes removed. Single-cell suspensions were prepared and restimulated with 50 μg/ml mBSA. Supernatants were harvested after 3 days and cytokine production was measured using TNFα/TNFβ assay kit from BD bioscience.

Antigen presentation assay was performed by incubating 2 × 10^6 WT or PLCγ2−/− immature DCs with ovalbumin-peptides as antigen. CD4+T cells purified from OT-II mice (which can only recognize ovalbumin) were labeled with CFSE (2 μM) and added to the plates at a final concentration of 1 × 10^6 cells/well. After 3 days, T cells were recovered, stained with an APC-conjugated CD4 antibody (BD Bioscience) and T cell proliferation was assessed by FACS analysis.

DC migration assay was performed using a competitive in vivo homing assay. DCs from WT or PLCγ2−/− mice were fluorescently labeled with high (2 μM) and low (0.5 μM) CFSE and coinjected in the ratio of 1:1 into each footpaw of recipient mice (4-8 mice per group). After 2 days recipient mice were sacrificed, draining popliteal lymph nodes were recovered and analyzed for presence of CFSE+ DCs for B and FACS. Time-lapse video microscopy was used to film DC:T cell interactions starting 10 minutes after addition of T cells to the DC culture, for a maximum of 30 minutes.

Results: In this study, we determined the inflammatory and osteolytic response of WT and PLCγ2 null mice to mBSA-induced arthritis, a model dependent on T cell activation. Unexpectedly, we found that PLCγ2 null mice were protected from bone loss and inflammation in this model of arthritis (Fig 1). Despite the ability of the PLCγ2−/− CD4+ T cells to be activated in vitro by PMA and ionomycin, confirming that that PLCγ2−/− T cells have no intrinsic functional or developmental defects, we found that the ex-vivo secretion of TNFα and IL-23 by activated CD4+ T cells in response to mBSA was impaired.

Since PLCγ2−/− mice are known to have defective B cell maturation, we injected WT CD45.1 congenic B cells in the tail vein of PLCγ2−/− mice and evaluated the inflammatory response to mBSA-induced arthritis after adoptive transfer. Interestingly, despite the presence of circulating mature B cells, PLCγ2−/− mice were still protected from mBSA-induced arthritis (total Local Disease Score for each footpaw) DCs in PLCγ2−/− mice completely rescued the inflammatory response. However, osteoclast recruitment and focal osteolysis in PLCγ2−/− mice receiving WT DCs were still impaired, further emphasizing the importance of PLCγ2 in OC development even under an ongoing inflammatory condition.

Finally, we turned to understanding how PLCγ2 modulated DC activation. We found that PLCγ2 did not alter DC maturation, but the molecule affected the capacity of the cells to migrate and interact with adjacent T cells. Thus, PLCγ2 deletion impacted DC trafficking to the lymph nodes in vivo and chemotaxis in vitro (87% and 73% reduction respectively from WT) as well as the capacity to embrace and activate T cells. Consequently, in vitro antigen presentation was defective in PLCγ2−/− DCs.

Conclusion: Our finding reveals that PLCγ2−/− mice are protected from bone loss and inflammation using the mBSA model of arthritis. We found an unexpected role for PLCγ2 in controlling DC function during T cell activation. In conclusion, our study demonstrates that PLCγ2 governs immune responses as well as OC formation, positioning the molecule as a possible therapeutic target for inflammatory and osteolytic diseases such as arthritis.

Figure: PLCγ2−/− mice are protected from inflammation and bone loss associated with m-BSA-induced arthritis.

Histological sections of WT and PLCγ2−/− mice following mBSA induced arthritis. Inflammation (stars), OC recruitment (red cells stained with TRAP) and bone resorption (arrows) are visible only in WT mice.

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