Creation of mixed bone marrow chimeras with appropriate controls

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Method Article

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Abstract

We report that IgA+ PC also can produce the anti-microbial mediators TNFα and iNOS which appears to arise in the unique environment of the gut, and may be critical to mount effective responses to microbial assault. To examine the functional relevance of TNFα/iNOS expression in IgA+ cells, we created mixed-bone marrow \(BM) chimeras from TNFα/iNOS double knockout mice \(dKO), in combination with B-cell deficient \(JH-/-), using JH-/- or Rag2-/- mice lethally irradiated recipients, thus creating mixed BM chimeras in which B cells are unable to produce TNFα/iNOS. Here we describe in detail the generation of these mixed bone marrow chimeras.

Introduction

We report that IgA+ PC also can produce the anti-microbial mediators TNFα and iNOS which appears to arise in the unique environment of the gut, and may be critical to mount effective responses to microbial assault. To examine the functional relevance of TNFα/iNOS expression in IgA+ cells, we created mixed-bone marrow \(BM) chimeras from TNFα/iNOS double knockout mice \(dKO), in combination with B-cell deficient \(JH-/-), using JH-/- or Rag2-/- mice lethally irradiated recipients, thus creating mixed BM chimeras in which B cells are unable to produce TNFα/iNOS. Here we describe in detail the generation of these mixed bone marrow chimeras.

Reagents

Sterile PBS \(Gibco) 26 gauge needles and 1cc syringes \(BD) 6 well tissue culture plates \(BD) Surgical instruments \(sharp, sturdy scissors for cutting through femur), medium forceps Kim wipes and bench diapers Alcohol spray bottle 75% ethanol Cell counting equipment \(trypan blue dye and hemocytometer) Clean pipetmen & sterile tips Sterile 15ml and 50ml Falcon tubes, Place a sterile PCR tube with a small hole on the bottom in to a sterile eppendorf tubes with 200ul of sterile PBS. 5ml Polystyrene tube with cell-strainer cap \(BD Biosciences). Ice bucket Sterile Red blood lysis buffer Neomycin Sulfate \(Sigma Aldrich)

Equipment

MDS Nordion Gammacell irradiator \(GC40E) Allegra 6R Centrifuge \(Beckman Coulter) Eppendorf centrifugue 5415D Hood

Procedure

RECIPIENTS: 1.Recipient mice \(Rag2-/- or JH-/-) should be in good health \(6-10 weeks old). We always sex-match donor and recipients. 2. Separate mice by groups to irradiate in a container. 3. Wipe down mouse container from irradiator with antiseptic and place the mice there. 4. We use a MDS Nordion Gammacell irradiator \(GC40E). The final dose is 1100 cGy divided in two cycles of 550cGy on the same

day. 3. Take mice back to colony for injection. DONOR CELLS: Harvesting bone marrow from donor mice \(WT, dKO, JH-/-) \(Need at least 1 million cells/recipient) 1. One mouse \(4 bones: femurs & tibias) should yield over 40-50 million cells. 2. Sacrifice donor mouse, spray down with ethanol and remove skin around legs. 3. Remove leg at hip joint \(can feel with fingers where to cut). 4. Remove skin and muscle by cutting skin off and using Kim wipes to “rub” off muscle. 5. Place bones in a well with PBS \(you can use a sterile 6 well plate or a petri dish). 6. Continue from now on in a biological safety cabinet and use sterile technique from now on. 7. Separate femur from tibia/fibula at the knee level. 8. Place them for a few seconds in a well with 75% of ethanol. 8. Rinse bones with fresh and sterile PBS twice. 9. Prepare a 1.5ml eppendorf tube with 200ul of PBS, put a PCR tube with a small hole on the bottom \(you can use a needle to make the hole) in to the eppendorf tube. 9. Cut ends of bone off and transfer to new dish containing PBS until it comes time to process them. 10. Place a femur/tibia together in to the tubes that you prepare on step 9. 11. Run a short spin in the eppendorf centrifuge to flush bone marrow cells. You will see all the cells in the 200ul of PBS at the bottom of eppendorf tube. 12. Lyse red blood cells with sterile lysis buffer. 13. Pass cell suspension through cell strainer to remove large fragments. 14. Count cells; resuspend them in PBS at a final concentration of 2 million/100ul. 15. For the mixed bone marrow chimeras we need to mix two different donor cells at a defined ratio before injection. 16. Store on ice until ready to inject. INJECTION: Tail vein procedure 1. Heat the recipient mice up first with the heat lamp until you can see the tail vein, make sure they do not get too hot. 2. Place mouse into restrainer with tail sticking out. Wipe injection site with alcohol 3. Inject 100ul cells \(1 million) i.v. through the tail vein of the recipient mice with a 26 gauge needle. Watch for clearing of vein lumen \(success). If not in vein, move more proximal until successful. Apply pressure to injection site. 4. Put them back in to their cages and MAINTAIN THEM ON ANTIOBIOTIC-WATER during two weeks \(filter 2g/L of Neomycin Sulfate in to water). 5. Analyze them after at least 6-8 weeks. CAUTION: All mixed BM recipients should be generated using the same cohort of recipient mice in order to maintain similar microbiota in the small intestine to be comparable.

Timing

The whole procedure should take about 8 hours.

Troubleshooting

If recipient mice die during the first 10 days after injection, it could be because of the following reasons: Donor cells don’t repopulate recipient’s bone marrow \(perhaps missed the i.v. injection). Sterile conditions during the procedure were not maintained, causing infection in the recipient mice.

Anticipated Results

We know that in our hands: - JH-/- + dKO mixed chimeric mice exhibited normal complement of immune cells in the periphery \(B cells, DC, T cells and neutrophils), although some changes in splenic microarchitecture were noted by histology. - JH-/- + dKO mixed BM chimeric mice exhibited a significant

drop in serum IgA but not IgG1 when compared with two groups of control mixed BM chimeric mice \(dKO + WT and JH-/- + WT mixed BM chimeric mice) and this decrease in serum IgA tracked with significant reductions in IgA+ cells within the LP as quantified by IF microscopy. - We noted that segmented filamentous bacteria \(SFB) were nearly absent from JH-/-+DKO mixed BM chimeras in small intestinal tissue. - iNOS/TNFα double-deficient mixed chimeras are more susceptible to infection with Citrobacter rodentium. - It is also important to note that in the dKO chimera \(JH-/- + dKO in to Rag2-/-) \(see Table. 1a), about 33% of the non-B cells lack iNOS and TNF, but we felt that the unperturbed levels of IgA in TNF+/- and iNOS+/- mice suggests haplosufficiency of these genes for IgA production. Nevertheless, a second cohort of chimeric mice can be important to confirm results \(see Table. 1b).

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