In a complete MHC mismatched allogeneic model of mouse bone marrow transplantation (BMT), naïve CD4+ T cells from the C57BL/6 donor spleen combined with T cell depleted bone marrow cells induce graft versus host disease (GVHD) in lethally irradiated BALB/C hosts, leading to death within 10-20 days. This is characterized by acute injury to the large bowel with severe diarrhoea. It has been reported that naïve CD4+CD62LloCD44hi T cells induce severe GVHD, but that effector memory CD4+CD62LloCD44hi T cells obtained from untreated nonnal donors do not induce GVHD in this model. We hypothesized that the poor GVHD-inducing capacity of effector memory cells from untreated donors may reflect their lack of previous exposure to host alloantigens. We tested this hypothesis by comparing the ability of effector memory T cells obtained from untreated donors and donors immunized to host alloantigens to induce GVHD. Donors were immunized by injecting 90 x10^6 spleen cells i.p. and after one week with 10 x10^6 cells. We sorted naïve (CD62Lhi CD44lo) and effector memory cells subsets sorted from unimmunized C57BL/6 donors. 100 days later, CD62LloCD44hi cells from immunized donors caused progressive weight loss and death in 100% of hosts (p <0.001). Whereas naive CD4+ T cells accumulated rapidly in the lymph nodes and spleen currently comparing the activity of memory CD4+ T cells to lethally irradiated BALB/C hosts. All mice were kept on antibiotic water (25 mg/ml neomycin /0.3 U/ml penicillin) supplemented with 10% heat-inactivated fetal bovine serum, 10 mM HEPES, 1% non-essential amino acids, 1 mM sodium pyruvate, 100 U/ml Penicillin + 100 μg/ml streptomycin. Growth of cultures was evaluated at the time points indicated by counting the total number of surviving cells and analyzing their distribution by flow cytometry. All experiments were performed with at least triplicate cultures. Results were assessed by labeling the cultures with 1mCi 3H-thymidine for the final 16 h of the 5 day incubation period. Data represent mean ± SD of triplicate values.

Results

Fig 1 Unimmunized and immunized spleenocytes express comparable levels of naive and effector memory CD4+ T cells

Fig 2 Effector memory CD4+ T cells from unimmunized C57BL/6 donors proliferate in response to BALB/c stimulators

Fig 3 Effector memory CD4+ T cells from unimmunized C57BL/6 donors induce GVHD

Fig 4 Histopathologic changes in the colon of lethally irradiated host seven days after transplantation of donor cells.

Conclusions

We found that CD62LloCD44hi effector memory CD4+ T cells from unimmunized donors failed to induce GVHD in almost all of the hosts over 100 days, whereas the effector memory CD4+ T cells from immunized donors caused progressive weight loss and death in 100% of hosts.

The naive CD4+ T cells home significantly better to mesenteric lymph nodes and colon while the effector memory CD4+ T cells from immunized donors show significantly increased accumulation in the liver compared to the effector memory CD4+ T cells from unimmunized donors.

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