IRCCS San Raffaele

UMG Dubium sapientiae initium

Evaluation of Lymphocytes in a Mice Model of Neuropathic Pain: Degranulation and Expression of μ Opioid receptor

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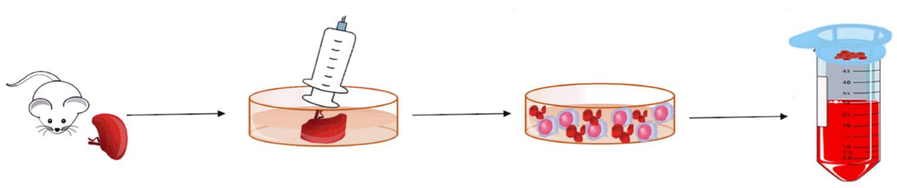
SUMMARY

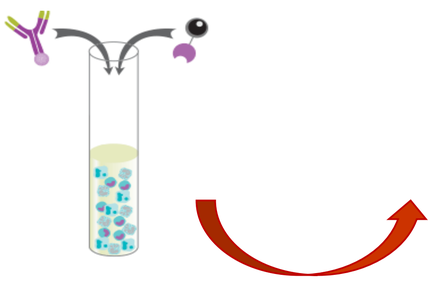
Pain is a significant clinical, social, and economic problem around the world, with physical consequences such as loss of mobility, loss of independence, and addiction to dependence on pain medications. Chronic pain is a serious disorder that declines in physical functioning and emotional distress. The lack of accurate diagnosis and appropriate treatment is one of the main limitations of pain in medicine. Rehabilitation programs are safe and effective in treating people with chronic pain. Recent studies described modulation of the percentage of B lymphocytes and NK cells expressing the μ opioid receptor as a potential marker for measuring pain. Neuropathic pain sufferers have decreased NK cell function, highlighting the need of further investigating the effect of opioid receptor expression on lympoid cells using mice models and defining their potential relevance as a pain monitor marker.

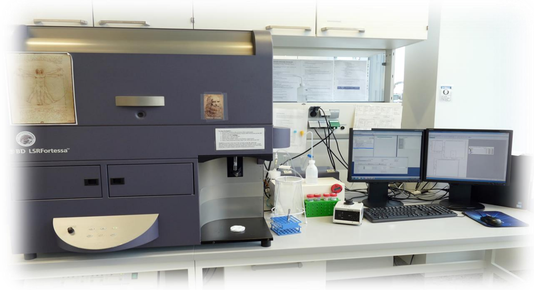
METHODS

1. Spleens from B6 mice cell suspension was filtered trough a nylon strainer and transferred into a centrifuge tube.



2. Staining with conjugated antibodies and aquisition using flow cytometry, Fortessa.

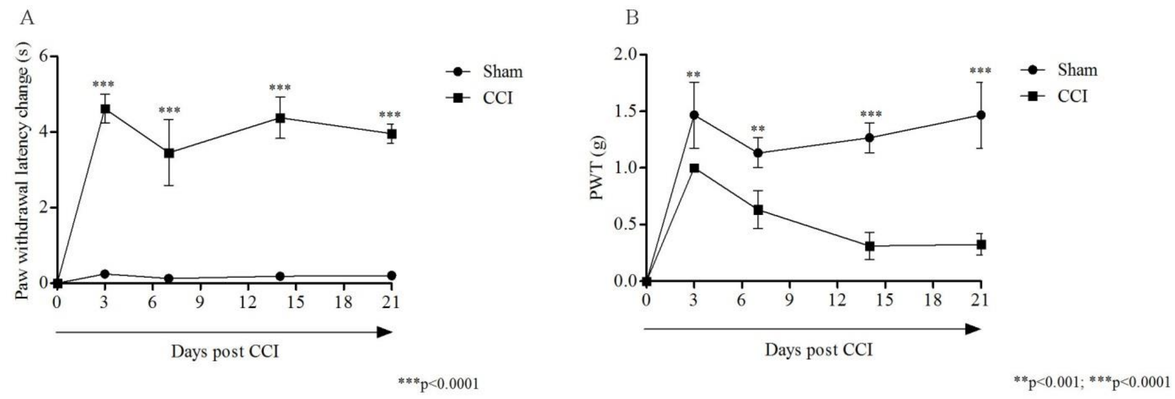




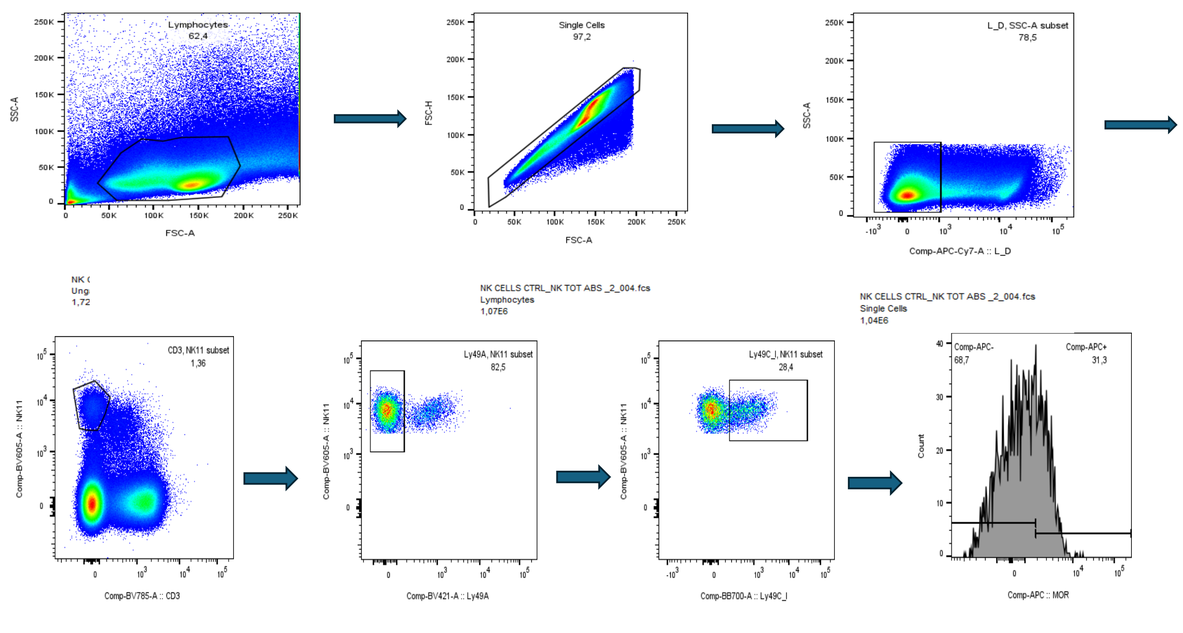
CONCLUSION

RESULTS

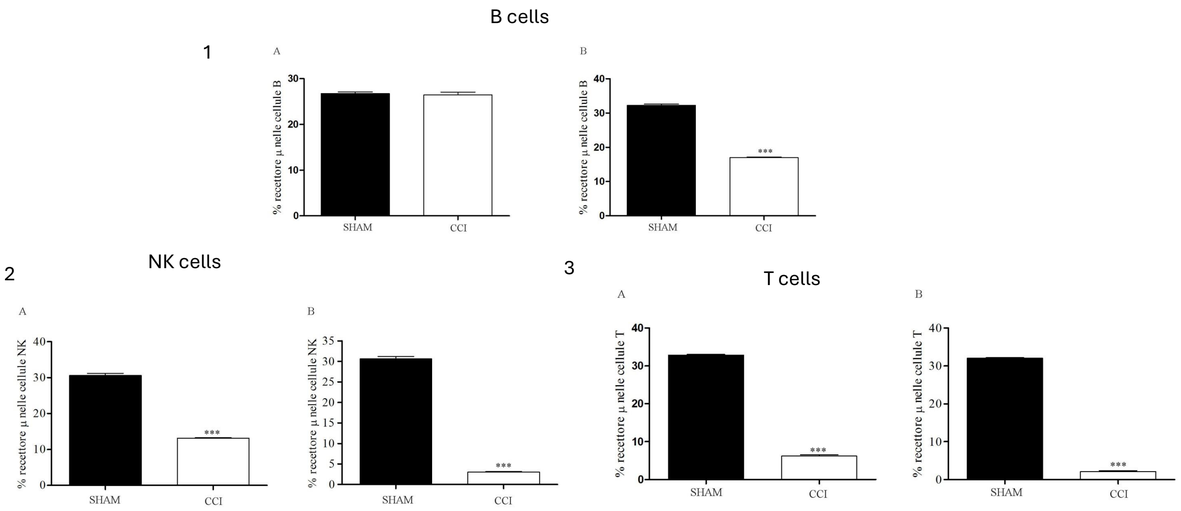
Effect of neuropathic pain in C57BL/6j mice on the latency of paw withdrawal after thermal stimulus (A) and the threshold of paw withdrawal after mechanical stimulus (B), assessed over a 21-day period. Each point represents the average of the shrinkage threshold (g) or shrinkage latency (s) calculated with SEM error for 10 mice. **p<0.001 vs sham; P<0.0001 vs Sham



Gating strategy of one representative experiment to study the expression of MOR in C57BL/6j mice lymphocytes



Flow cytometric analysis to measure MOR expression on B, NK and T lymphocytes from C57BL/6j mice 14 days after CCI (Figure 1,2,3 A) and 21 days after CCI (Figure 1,2,3 B). Results are expressed as a mean± SEM for 10 mice. p<0.0001 vs sham.



We observed that CCI causes a significant reduction in both mechanical withdrawal thresholds and thermal withdrawal latencies of the ipsilateral hind paw, indicating the presence of allodynia and thermal hyperalgesia as measured employing the Von Frey and the Hargreaves method respectively. Injection of vehicle (saline) did not change the mechanical withdrawal threshold or thermal withdrawal latency. No effects on contralateral paw withdrawal were found. Moreover, in the flow cytofluorimetry acquisition, we discovered that allodynia and thermal hyperalgesia evaluated in CCI mice correspond to a modification of percentage MOR-positive NK, B, and T cells compared to sham mice. CD107a cell surface assays revealed NK degranulation at 14 and 21 days following CCI. Furthermore, at the same time points, IFNg levels were seen to be modulated in both NK and T cells, with or without PMA/Ionomycin activation. Opioid receptors expressed on NK, B and T cells are a possible valid candidate for objective monitoring of pain. Preclinical studies will allow us to investigate the mechanism underlying the expression of opioid receptors μ as a marker of pain and to evaluate whether this marker is for "predisposition to pain". Here, we found that CCI mice present a significant lower percentage of Mu+ B, NK and T cells compared to the sham group at 14 and 21 days. These data correlate with NK degranulation and IFNg modulation according with the lymphocyte exhaustion.

Our results support the hypothesis that modulation of the μ opioid receptor on lymphocytes could become a valid method for pain measurement, helping to establish an innovative approach for a tailored therapeutic and rehabilitation program.

Acknowledgemtents

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