



Mesenchymal Stem Cells: a novel therapeutic approach for neurological diseases

Antonio Uccelli

Neuroimmunology Unit

Department of Neurosciences Ophthalmology and
Genetics

University of Genoa



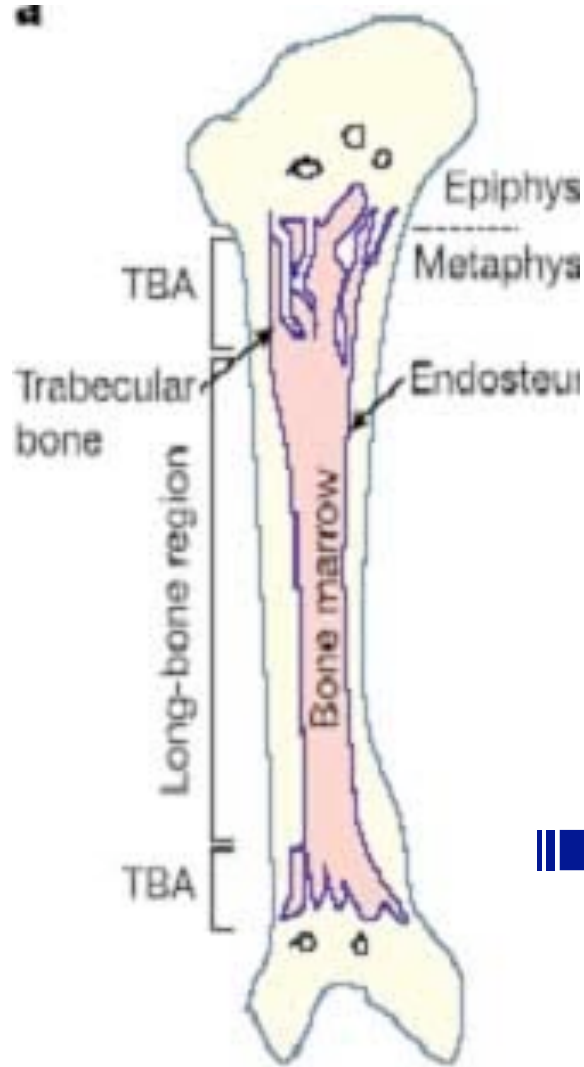
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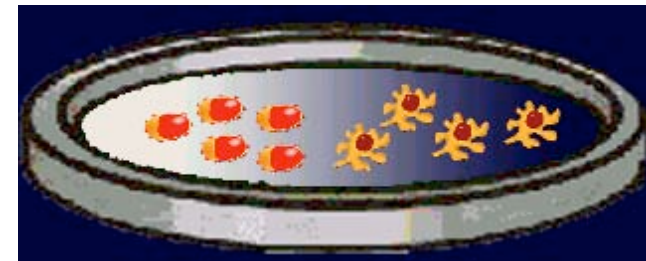


VIII Corso Residenziale di Neuroimmunologia,
Bergamo 8-11 Marzo 2006

Cellule staminali del midollo osseo



Cellule staminali ematopoietiche



Cellule staminali mesenchimali

Pluripotency of mesenchymal stem cells derived from adult marrow

Yuehua Jiang^{*†}, Balkrishna N. Jahagirdar^{*†‡}, R. Lee Reinhardt[§], Robert E. Schwartz^{*}, C. Dirk Keene^{||}, Xilma R. Ortiz-Gonzalez^{||}, Morayma Reyes^{*}, Todd Lenvik^{*}, Troy Lund^{*}, Mark Blackstad^{*}, Jingbo Du^{*}, Sara Aldrich^{*}, Aaron Lisberg^{*}, Walter C. Low^{||}, David A. Largaespada[¶] & Catherine M. Verfaillie^{*‡}

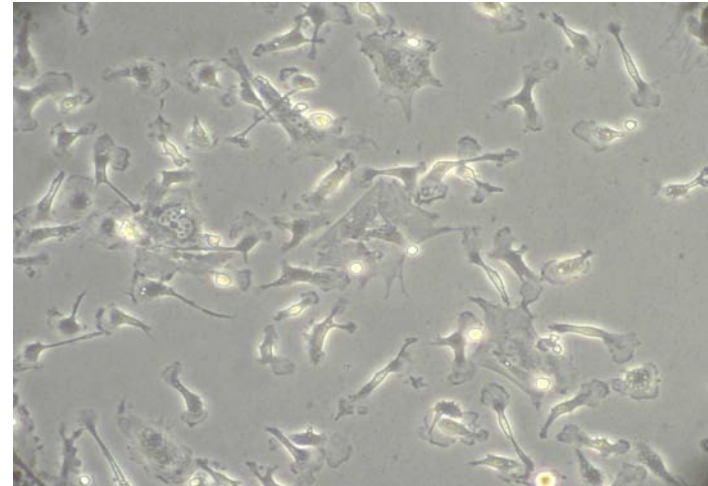
^{*} Stem Cell Institute, [‡] Division of Hematology, Oncology and Transplantation, Department of Medicine, [§] Department of Microbiology, Center for Immunology, ^{||} Department of Neurosurgery, and [¶] Department of Genetics, Cell Biology and Development, University of Minnesota Medical School, Minneapolis, Minnesota 55455, USA

[†] These authors contributed equally to this work

We report here that cells co-purifying with mesenchymal stem cells—termed here multipotent adult progenitor cells or MAPCs—differentiate, at the single cell level, not only into mesenchymal cells, but also cells with visceral mesoderm, neuroectoderm and endoderm characteristics *in vitro*. When injected into an early blastocyst, single MAPCs contribute to most, if not all, somatic cell types. On transplantation into a non-irradiated host, MAPCs engraft and differentiate to the haematopoietic lineage, in addition to the epithelium of liver, lung and gut. Engraftment in the haematopoietic system as well as the gastrointestinal tract is increased when MAPCs are transplanted in a minimally irradiated host. As MAPCs proliferate extensively without obvious senescence or loss of differentiation potential, they may be an ideal cell source for therapy of inherited or degenerative diseases.

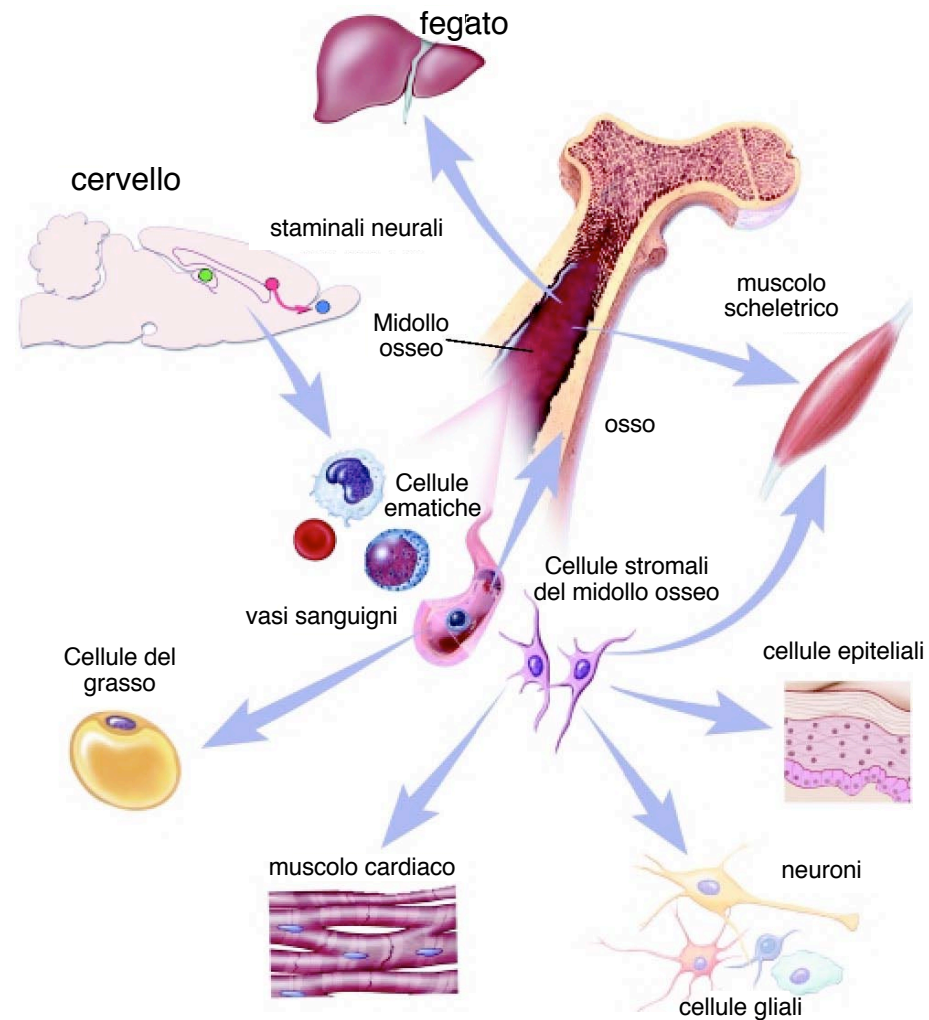
Cellule Staminali Mesenchimali (MSC)

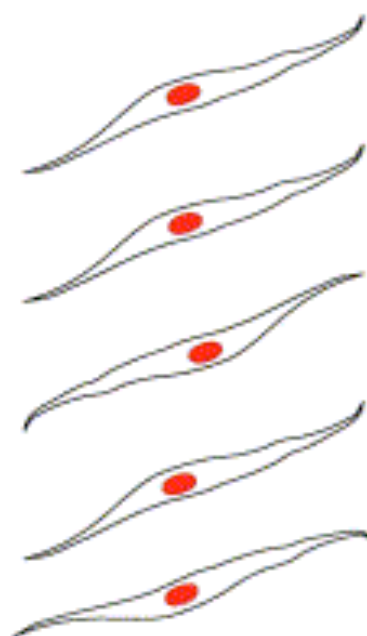
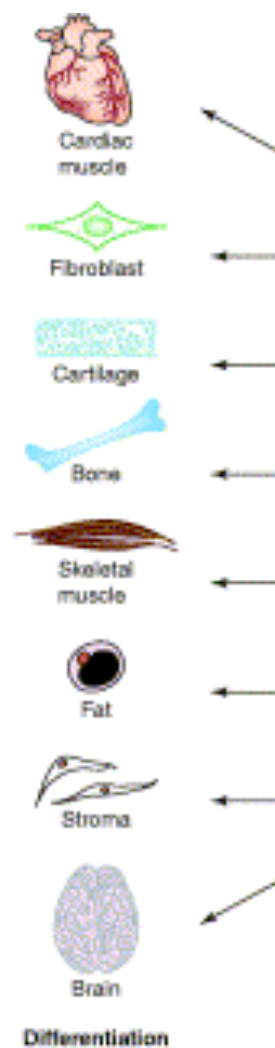
- E' una sottopopolazione di cellule che costituisce l'impalcatura del midollo osseo e regola il processo dell' Ematopoiesi.
- Vengono isolate dallo stroma del midollo osseo e si presentano come cellule aderenti, clonogeniche e dall'aspetto fibroblastico.
- La loro caratteristica di cellule aderenti, al contrario della maggior parte delle cellule del sistema ematopoietico, è alla base della selezione *in vitro* che prevede l'utilizzo di terreni selettivi per dar luogo alle colture.
- Dal punto di vista fenotipico le cellule staminali mesenchimali esprimono CD 9, Sca-1, CD 44 e sono negative per marcatori ematopoietiche (CD45, CD14)

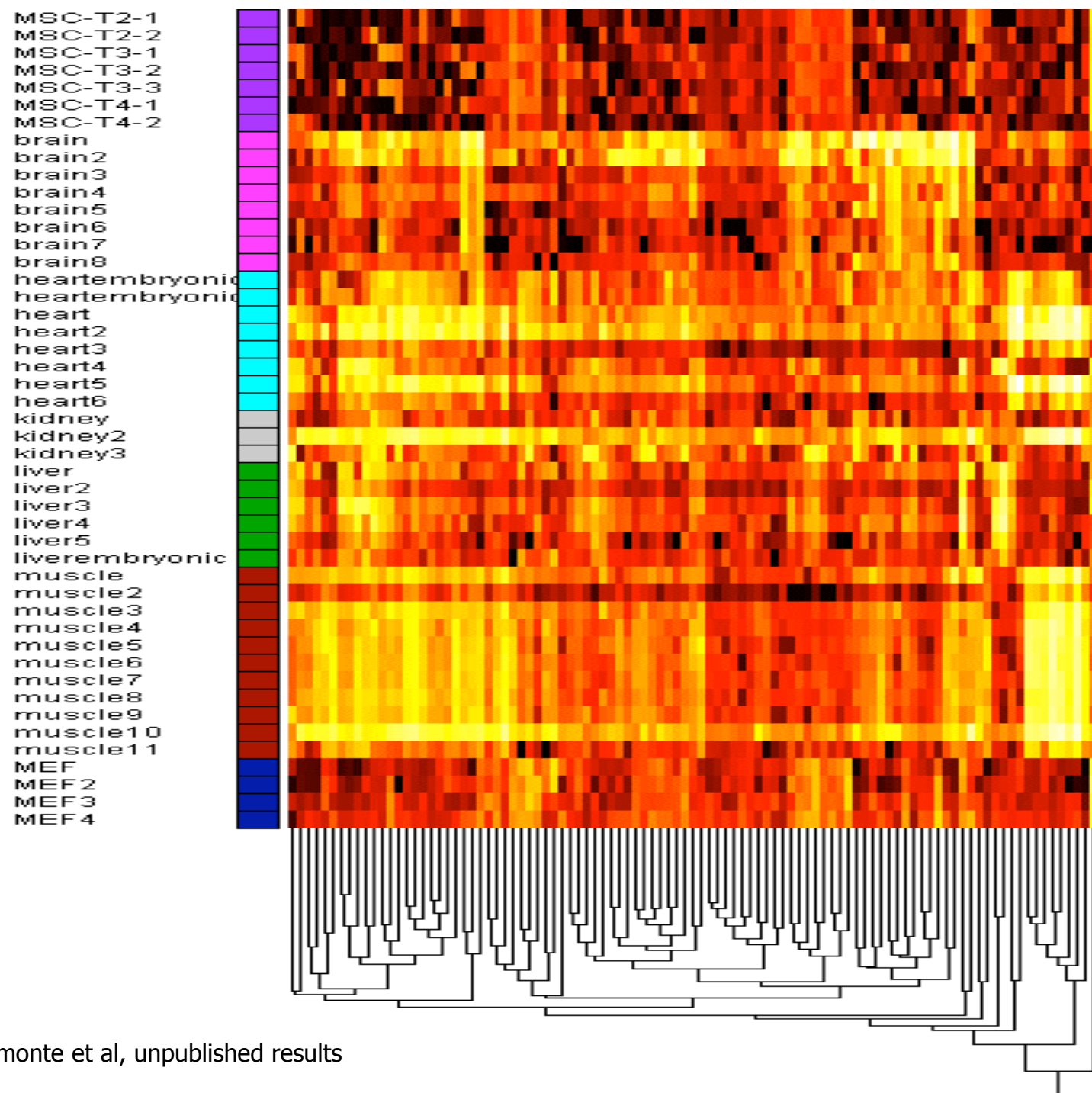


Le cellule staminali adulte

la transdifferenziazione

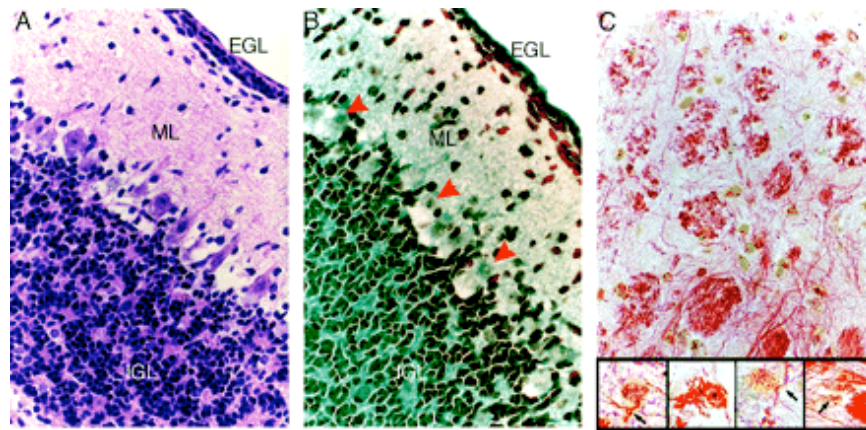
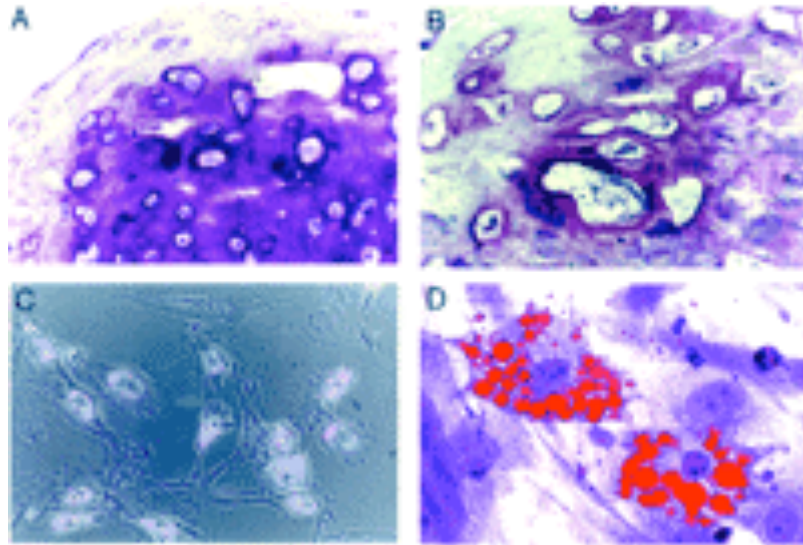






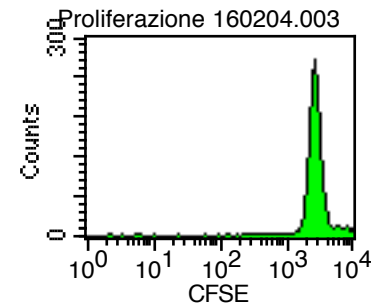
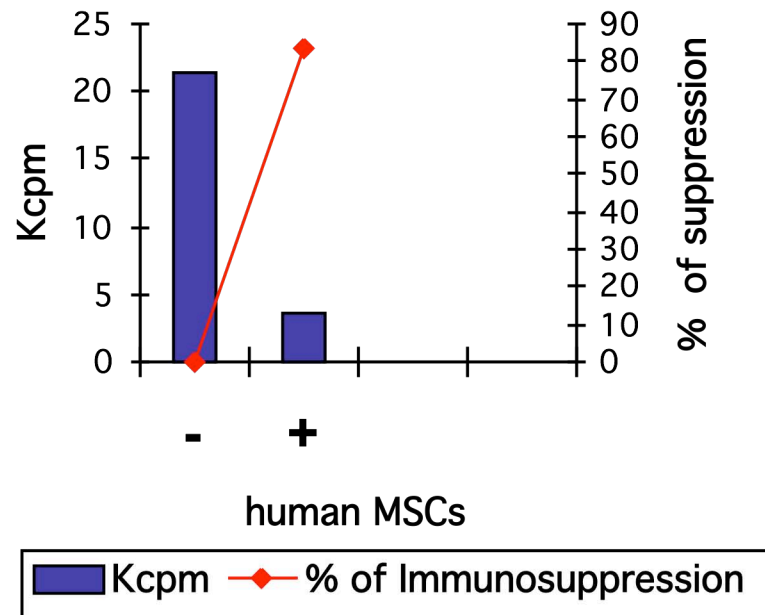
Pedemonte et al, unpublished results

Mesenchymal stem cells

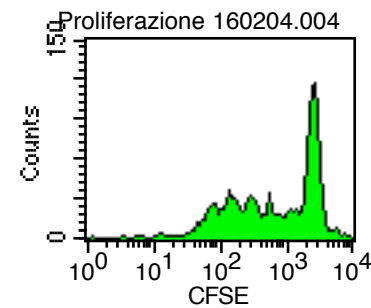


- Differentiate into multiple cells derived from the three germs layers (Pittinger *et al*, 1999)
- Upon tissue injury MSC may target the wounded brain under the influence of chemokines attempting to repair the damage (Wang *et al*. 2002)
- Suppress T cells activation (Di Nicola *et al*, 2002; Bartholomew *et al*, 2002, Krampera *et al*, 2002)

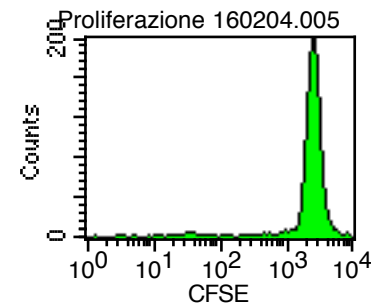
Inhibitory effect of human MSCs on T-cell proliferation



Unstimulated T cells



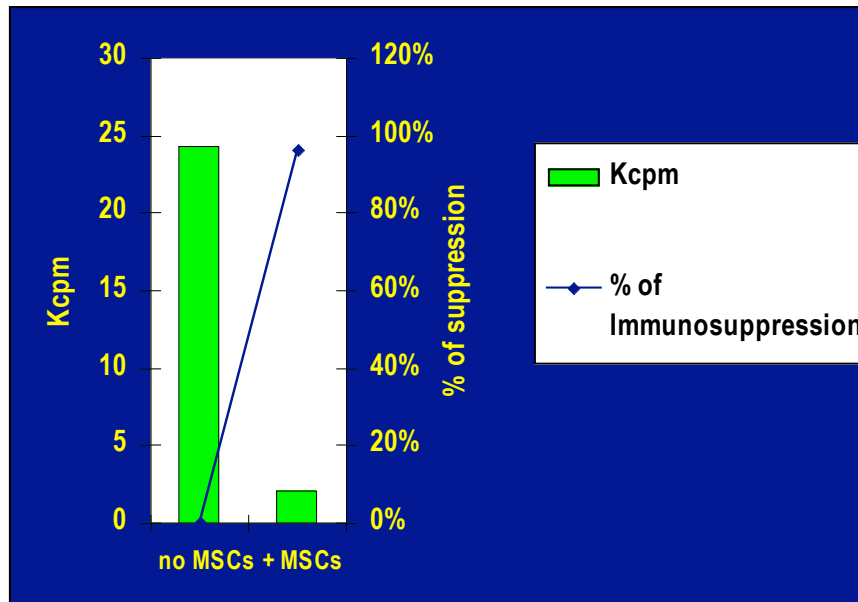
Anti CD3 - stimulated T cells



Anti CD3 - stimulated T cells + MSC

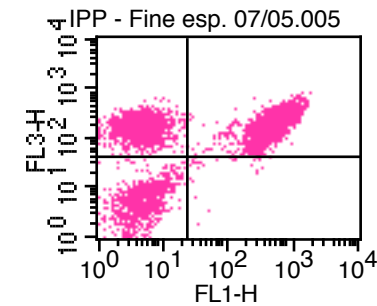
CFSE

Immunosuppressive effects of Human MSCs on *in vitro* IPP – activated $\gamma\delta$ T cells

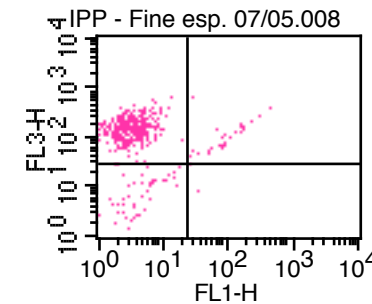


CD3

no MSCs



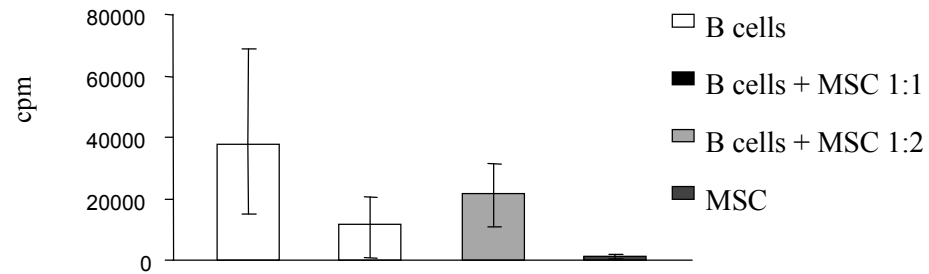
+ MSCs



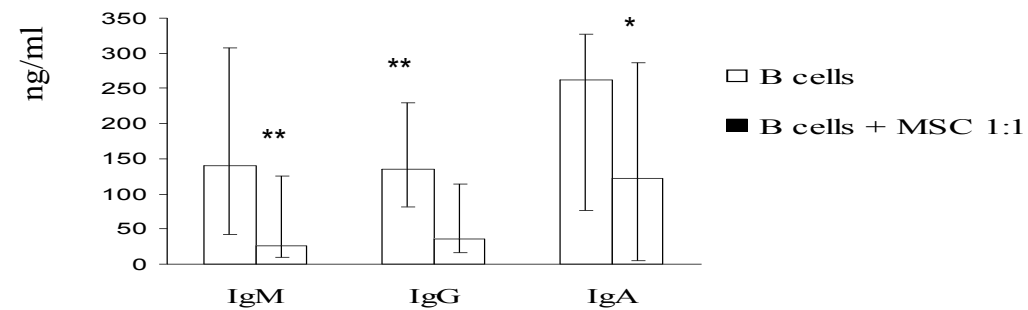
Vdelta 2

Collaboration with L. Battistini

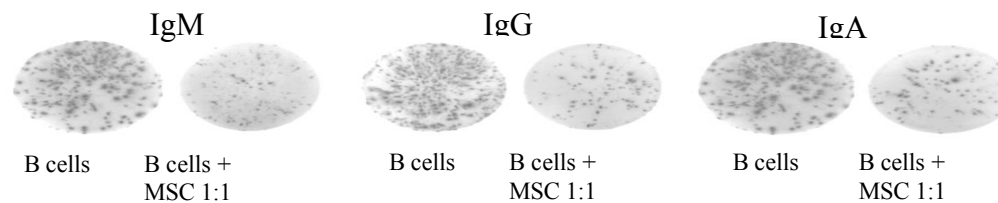
Le CSM inibiscono i linfociti B



Inibizione della proliferazione



Inibizione della produzione di anticorpi



Inibizione della differenziazione a plasmacellule

MSC for EAE

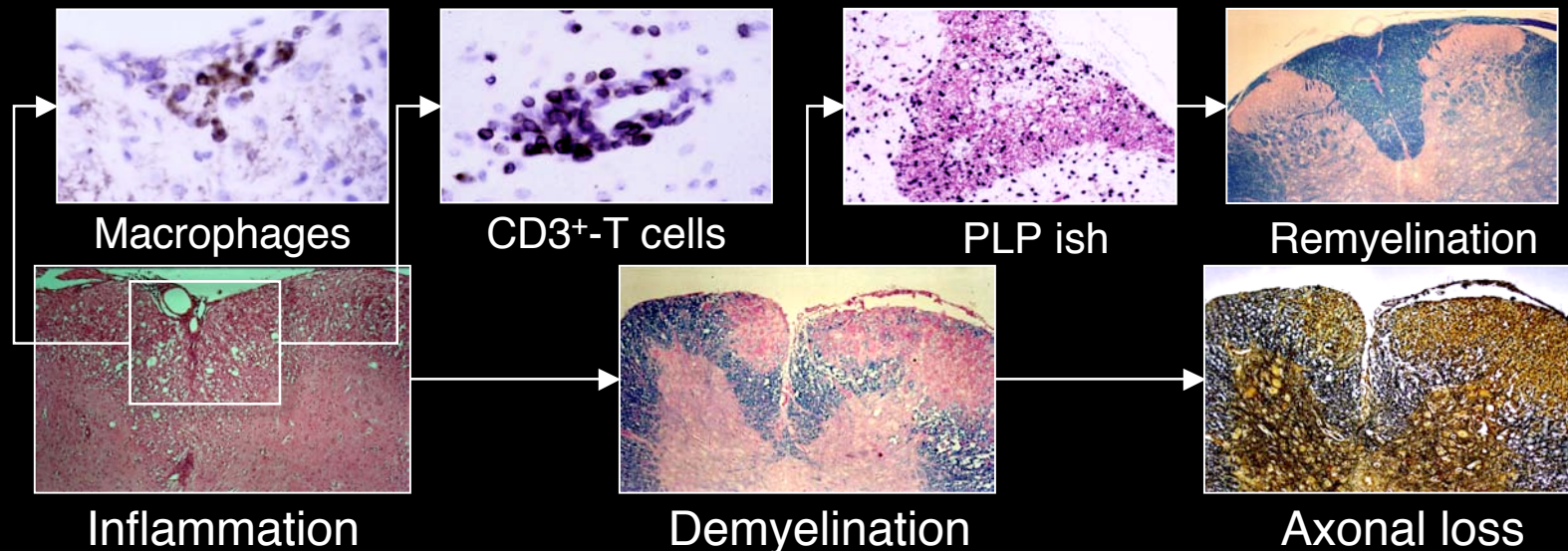
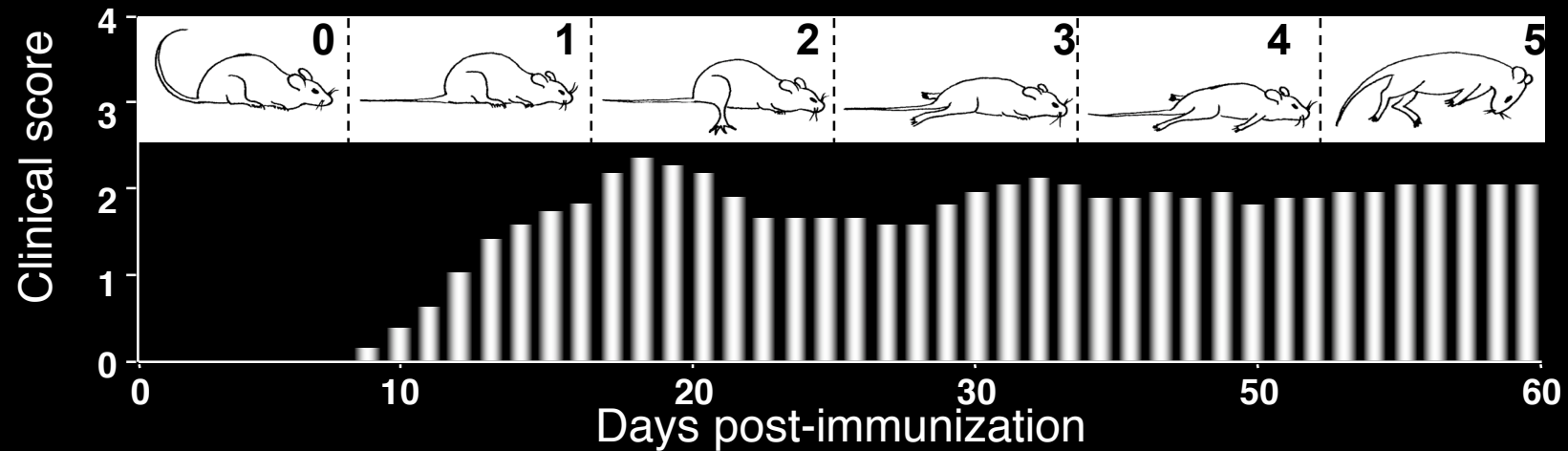
Do MSC affect the autoimmune
response?

Do they halt tissue destruction and
foster brain repair?

MOG₃₅₋₅₅-induced EAE in C57BL/6 mice

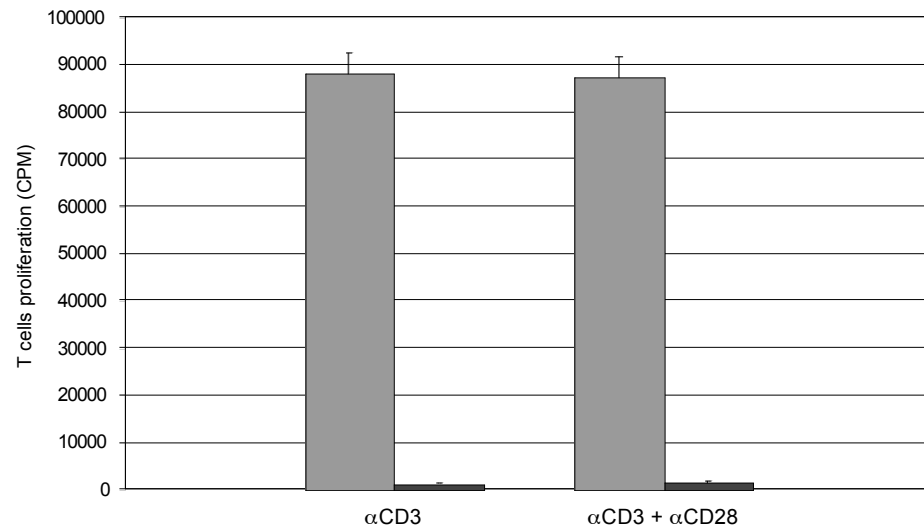
Active immunization: by s.c. injection of FCA, pertussis toxin and myelin antigens

Passive transfer: by i.v. injection of activated CD4⁺-Th1 myelin-specific T cells

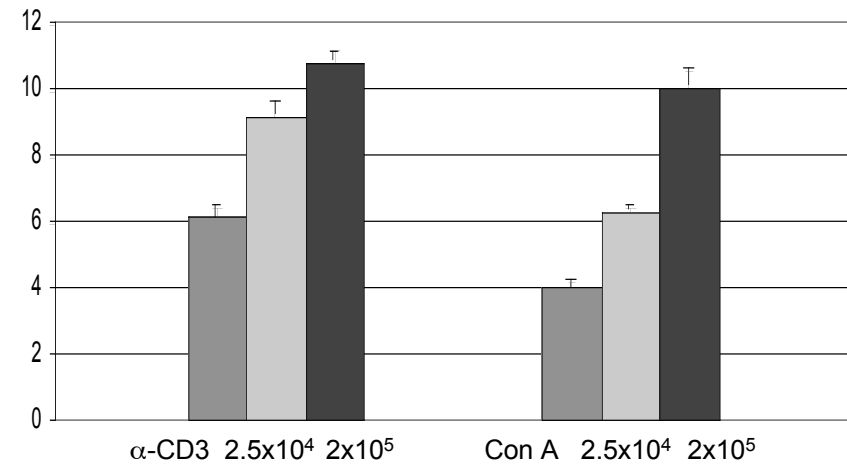
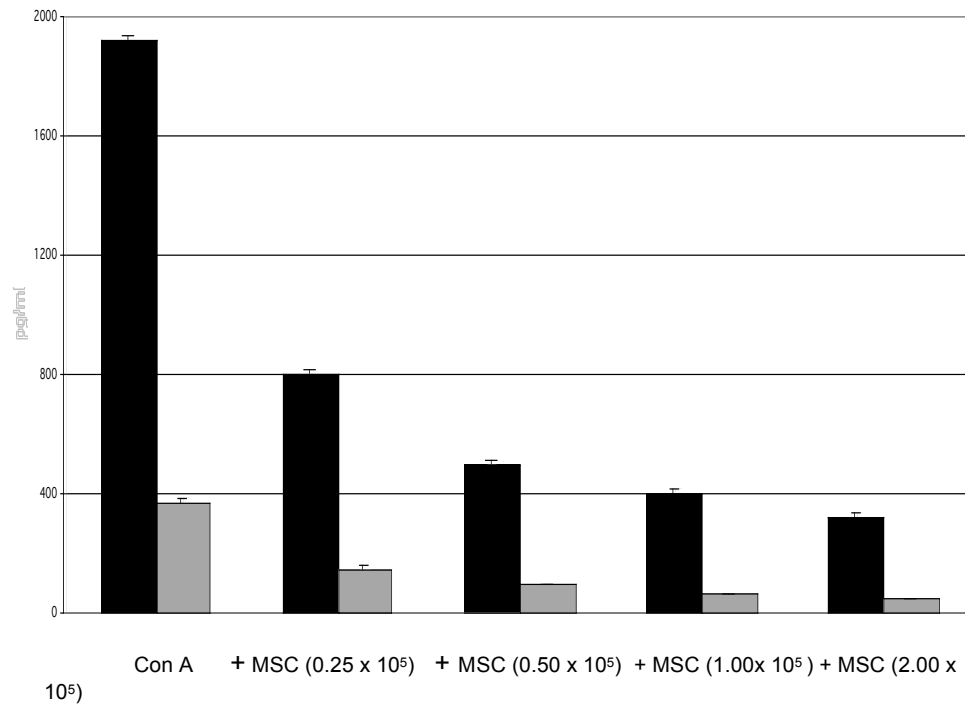
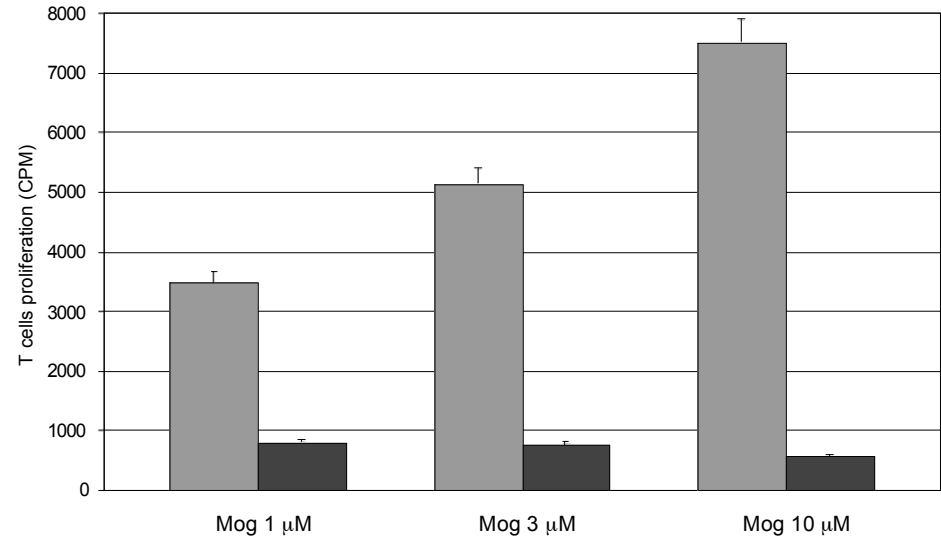


mMSC inhibits T cell proliferation

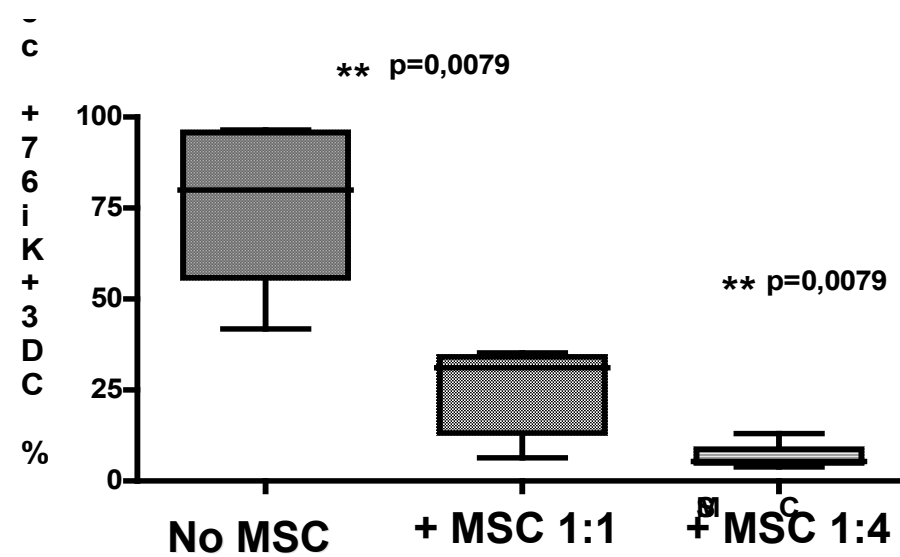
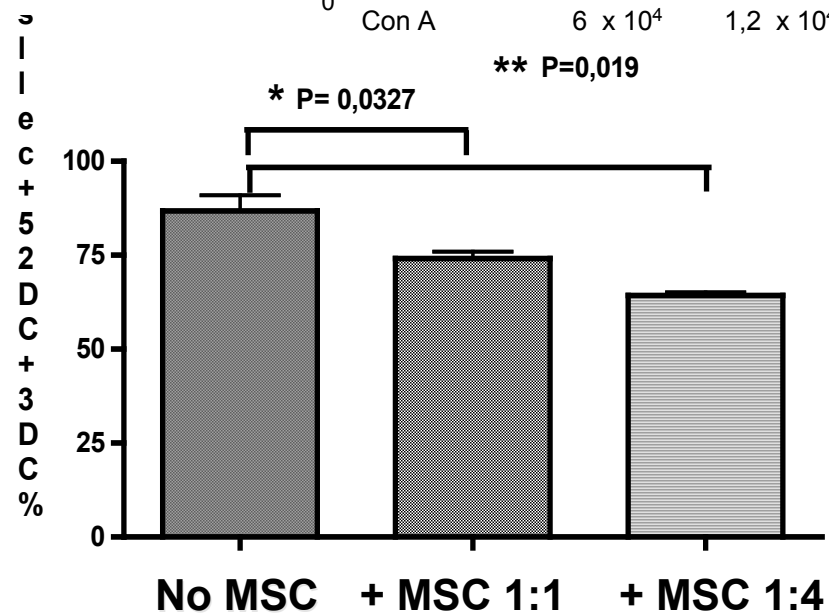
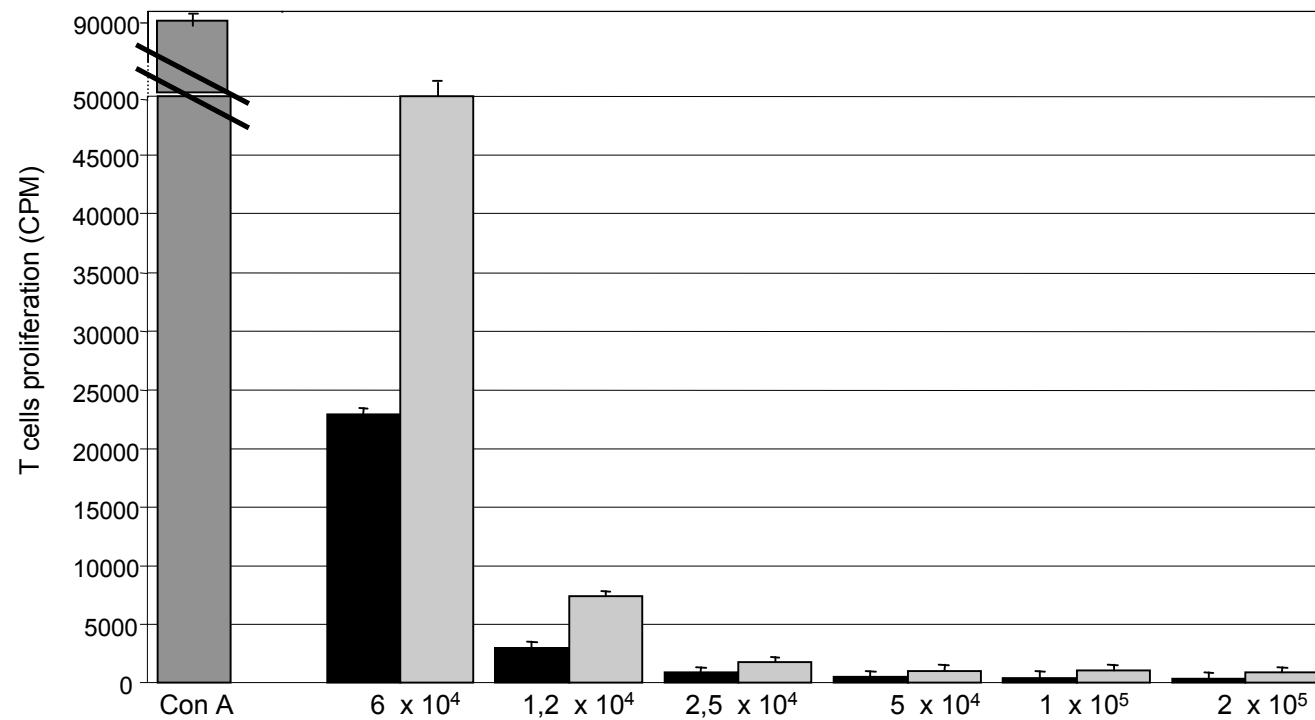
α CD3+ α CD28



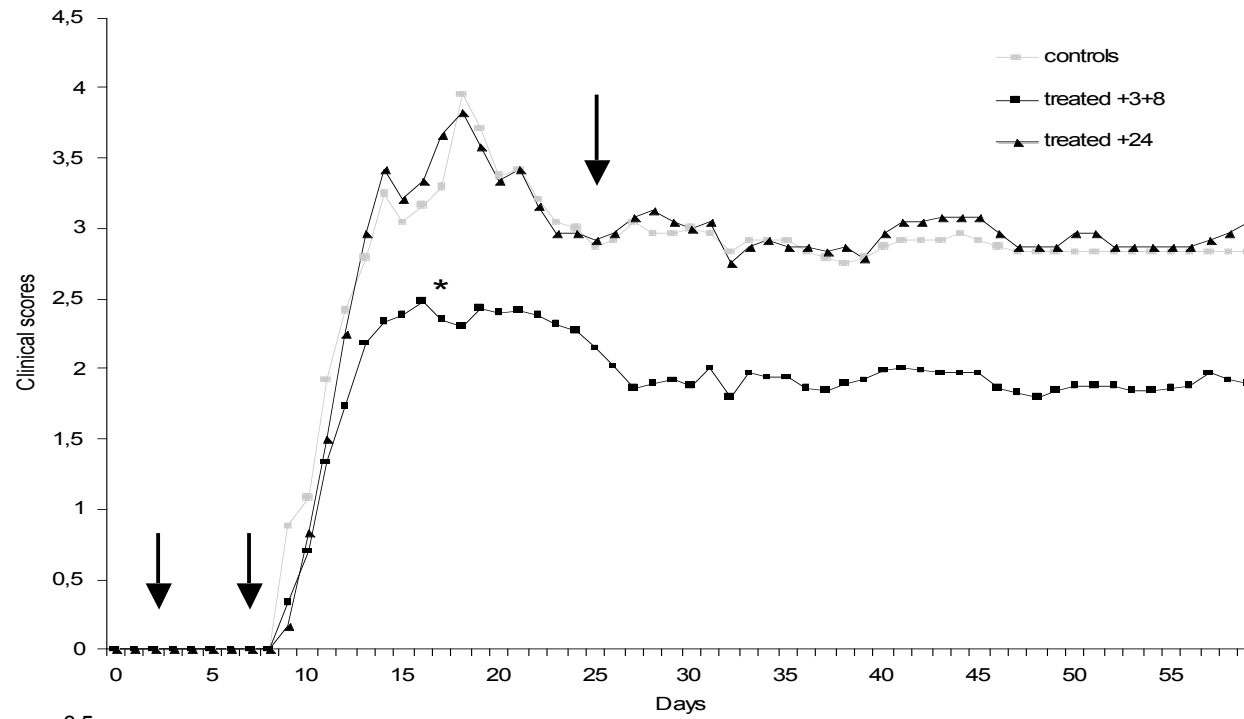
MOG



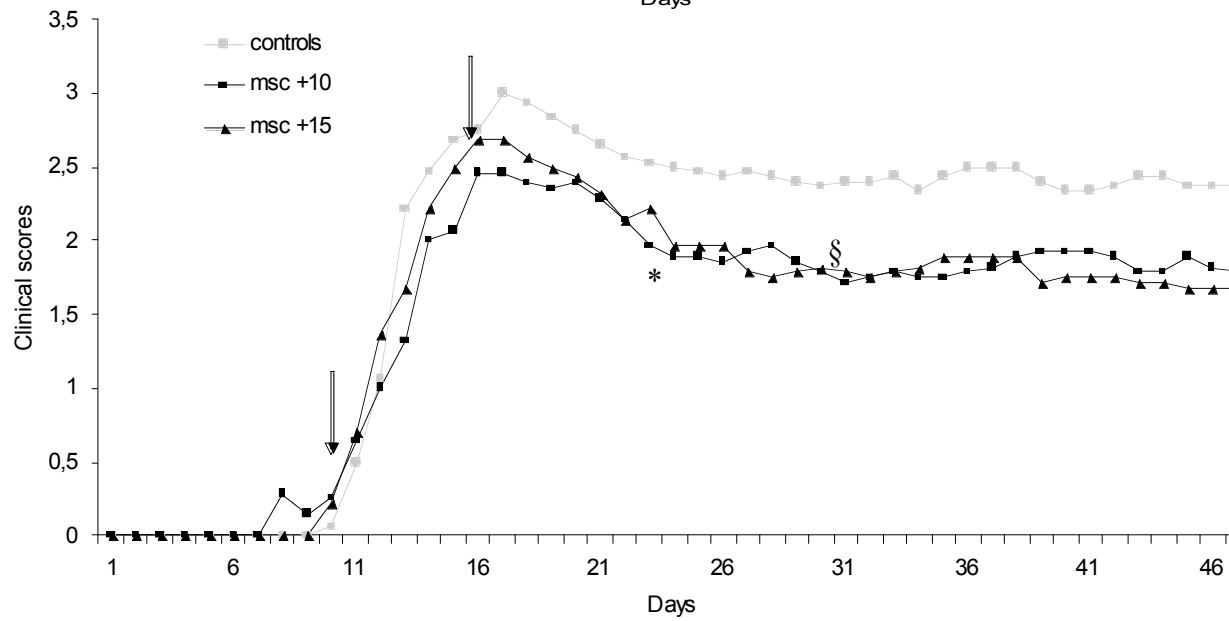
Zappia et al, Blood 2005



5a

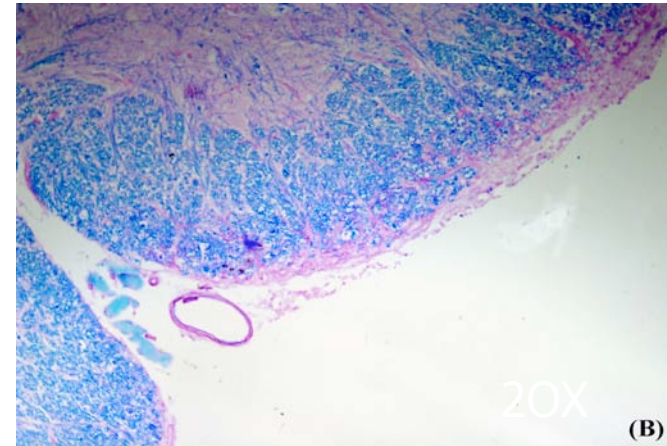
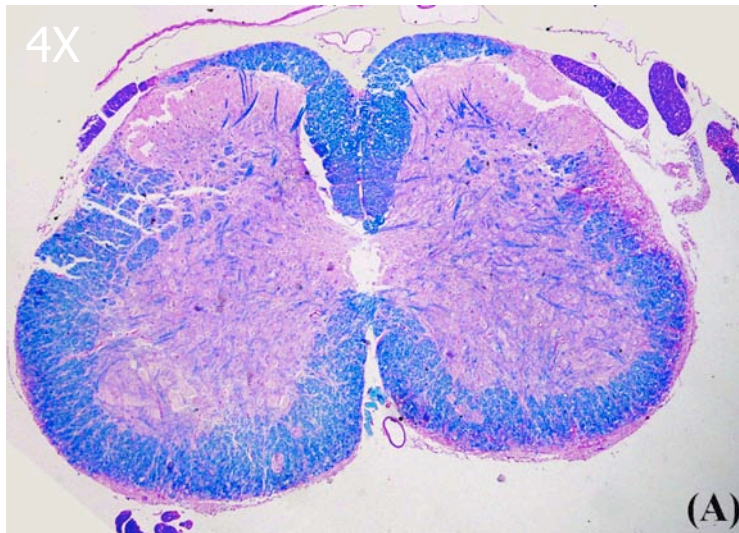


5b

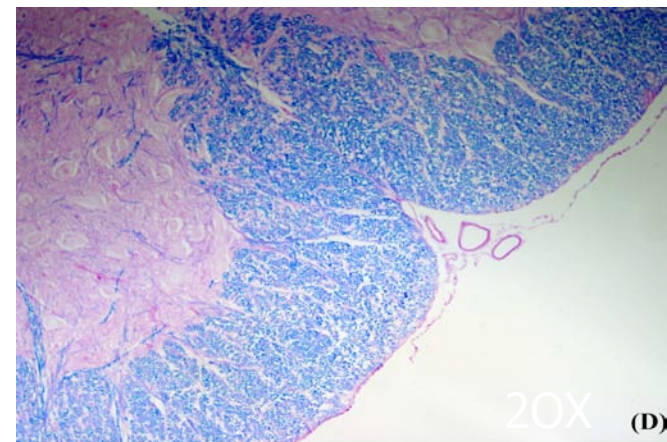
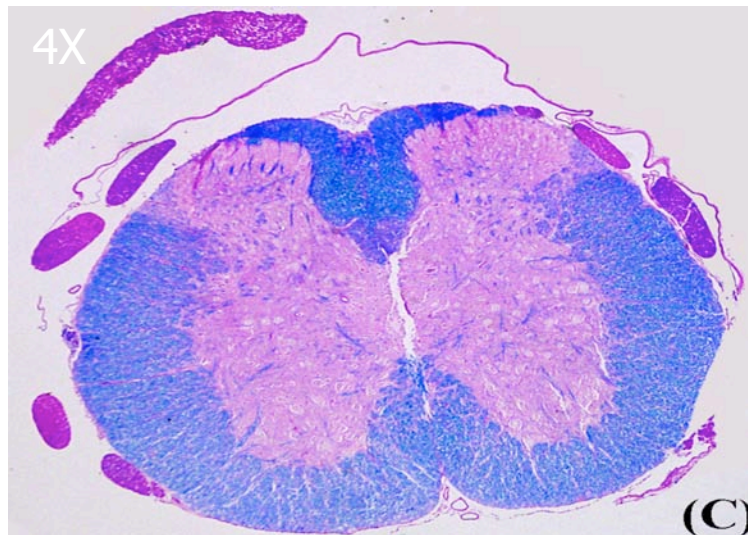


MSC reduces demyelination

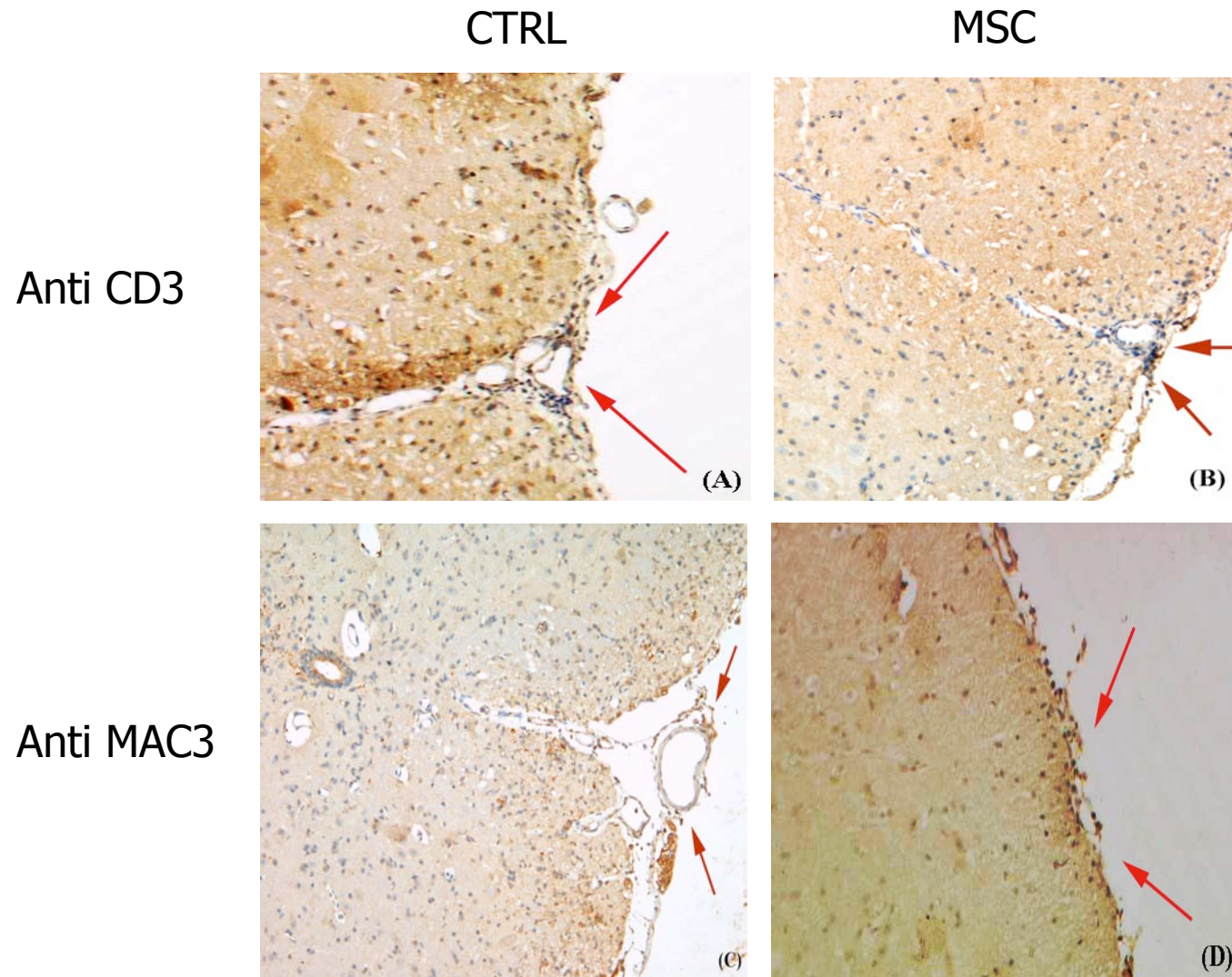
CTRL



MSC

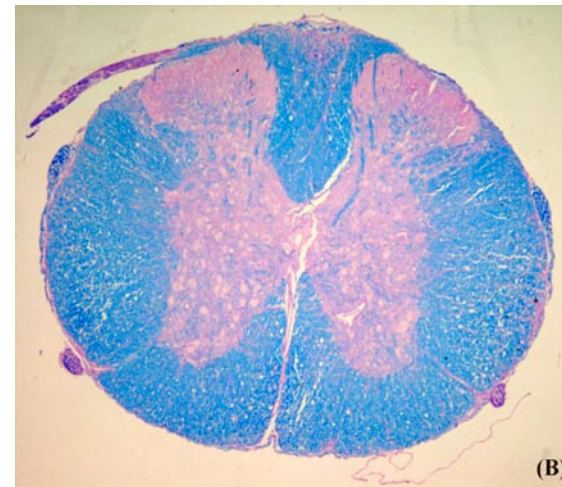
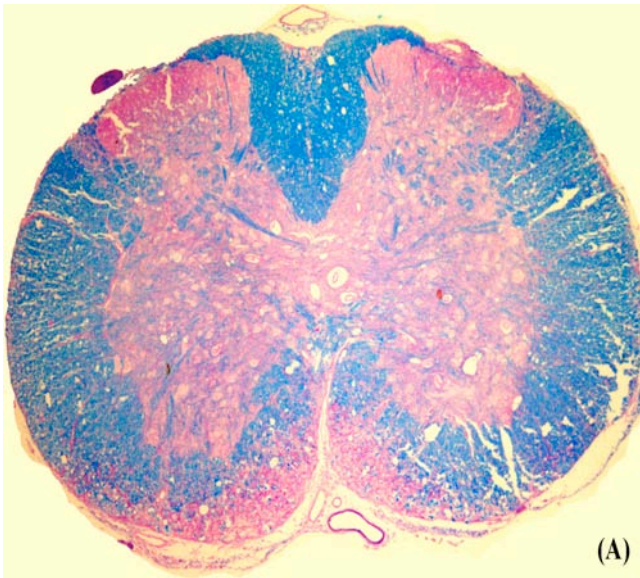


MSC reduces T cell and macrophage infiltration

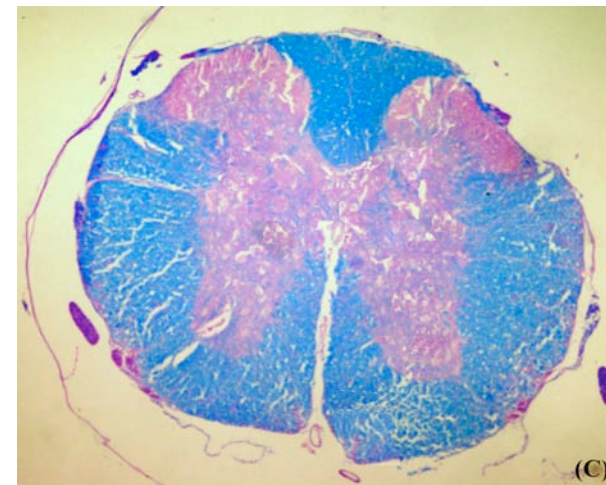


Therapeutic protocol

CTRL



MSC + 10



MSC + 15

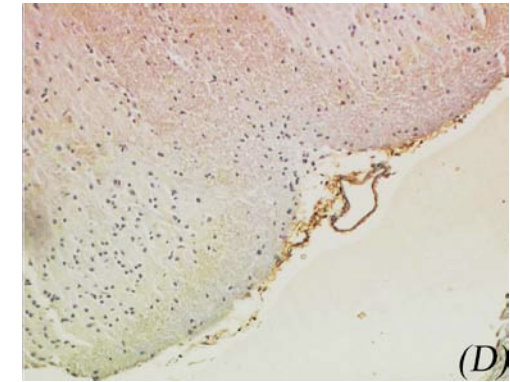
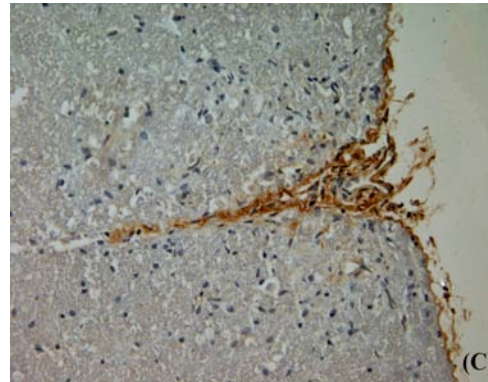
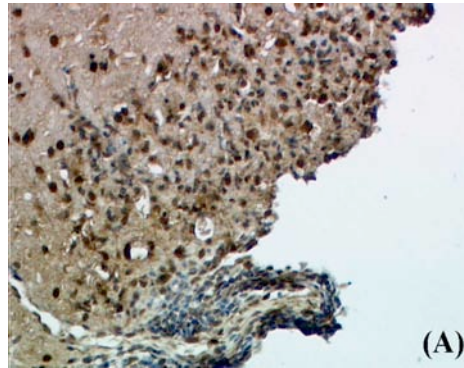
Therapeutic protocol

CTRL

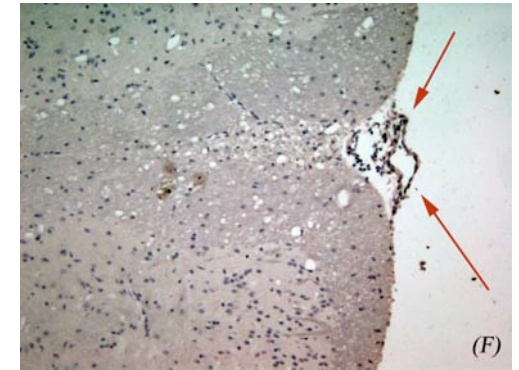
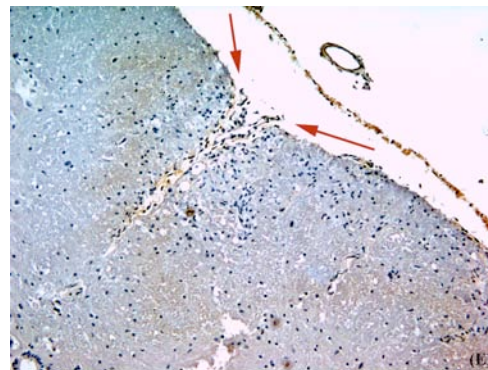
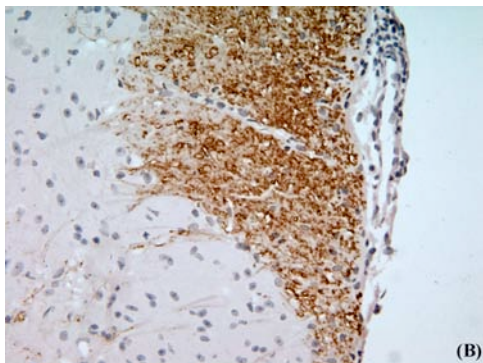
MSC +10

MSC +15

Anti CD3



Anti MAC3



	Disease incidence (%)	Disease onset (range)	Mean maximum neurological score (range)	Cumulative disease score	Demyelination Score	Macrophages (range)§	CD3+ cells (range)§
Controls	6/6 (100)	9,8 ± 1,0 (9 - 11)	3,9 ± 0,9 (2 - 5)	149,0 ± 46,8	3,8 ± 1,3 (2-6)	94,8 ± 34,5 (40–190)	146,2 ± 35,7 (71–200)
Treated +3+8	15/15 (100)	10,1 ± 1,2 (9 - 13)	3,1 ± 1,1 (1 - 5)	101,2 ± 48,6‡	1,9 ± 1,2‡ (1-5)	52,7 ± 27,2‡ (20–134)	54,7 ± 21,3‡ (22–103)
Treated +24	6/6 (100)	10,0 ± 0,9 (9 - 11)	4,0 ± 0,6 (3 - 5)	150,7 ± 49,7	3,2 ± 1,4 (1-6)	89,9 ± 26,9 (42–132)	107,7 ± 25,4 (73–155)
Controls	8/8 (100)	10,4 ± 0,6 (9 - 12)	3,2 ± 0,4 (2,5 - 4)	122,8 ± 17,8	6,1 ± 1,86 (2-8)	110,0 ± 21,9 (65–138)	123,8 ± 22,0 (71–158)
Treated +10	7/8 (88)	9,7 ± 1,8 (7 - 14)	2,8 ± 0,3 (2 - 3,5)	98,1 ± 14,3†	2,5 ± 1,3‡ (1-5)	52,7 ± 15,0‡ (34–87)	74,7 ± 17,4‡ (51–112)
Treated +15	7/8 (88)	10,3 ± 0,9 (9 - 12)	2,8 ± 0,3 (2 - 3,5)	94,2 ± 15,3	3 ± 2,2† (1-8)	66,9 ± 12,7‡ (48–88)	84,9 ± 19,9‡ (58–117)

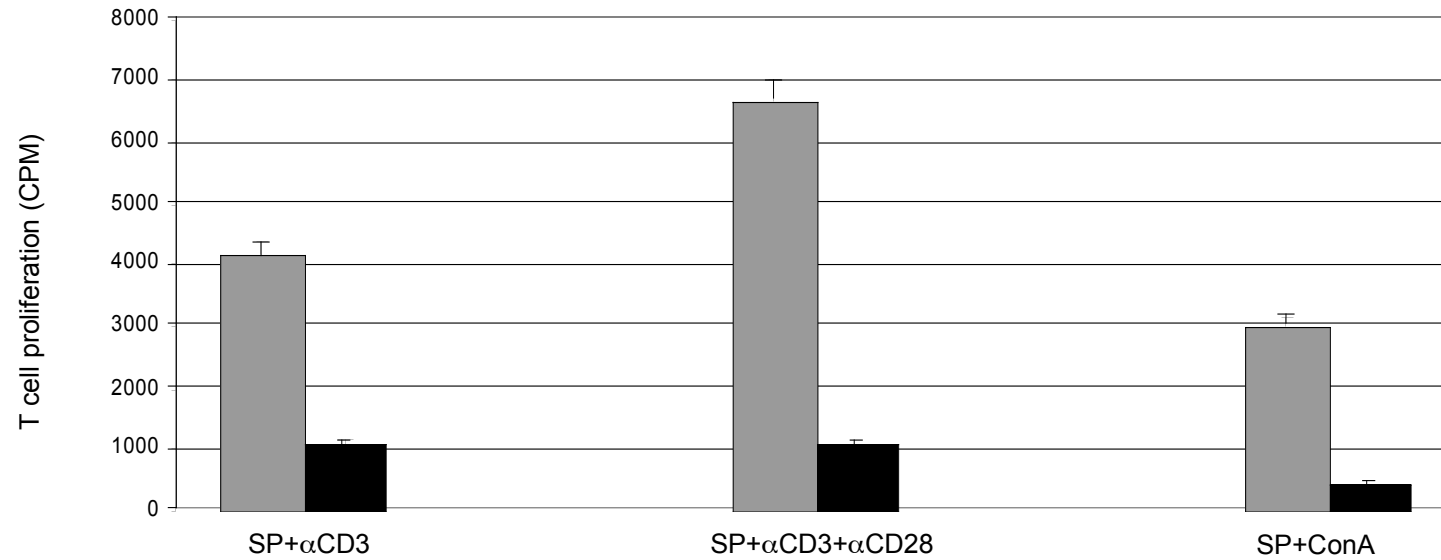
† p<0.05 (Mann-Whitney test)

‡ p<0.01 (Mann-Whitney test)

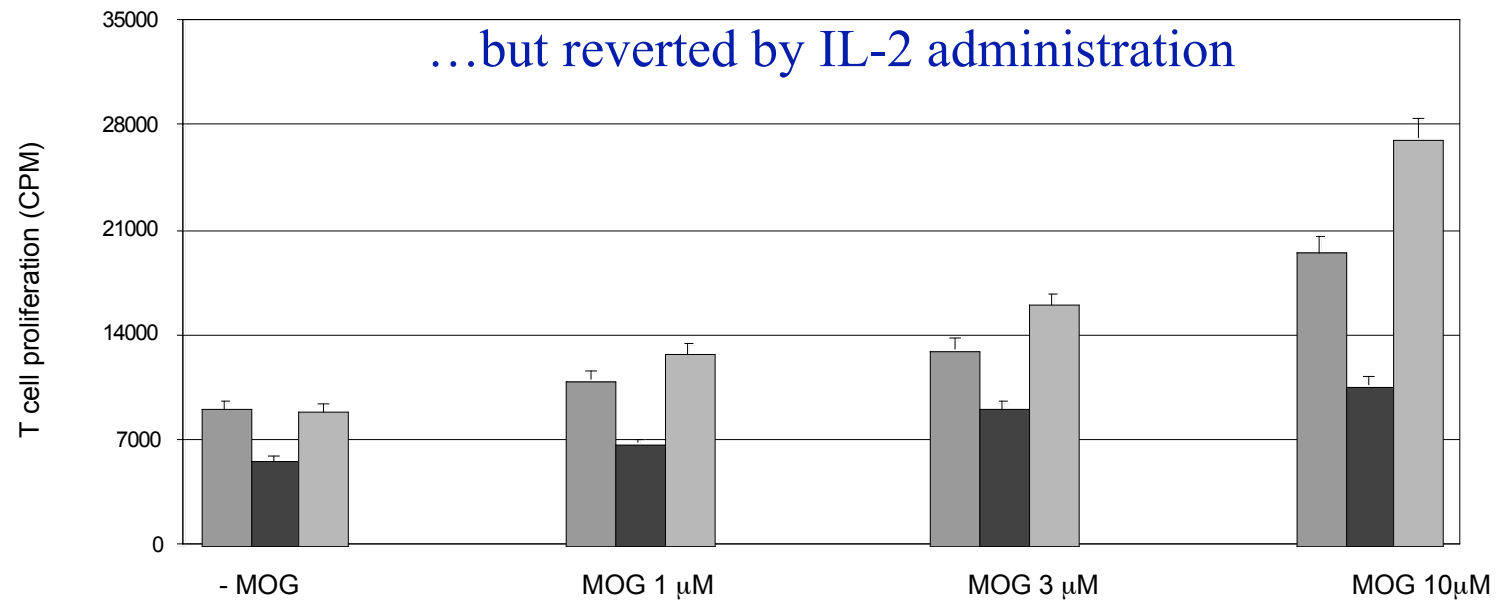
§ cells/mm²

Proliferation of T cells from mMSC treated mice is inhibited

8a

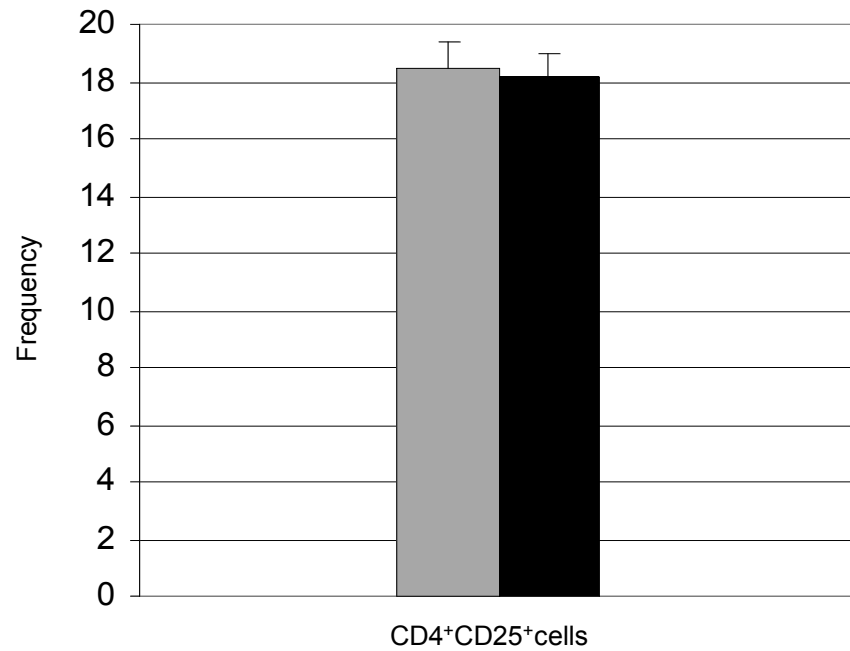


8b

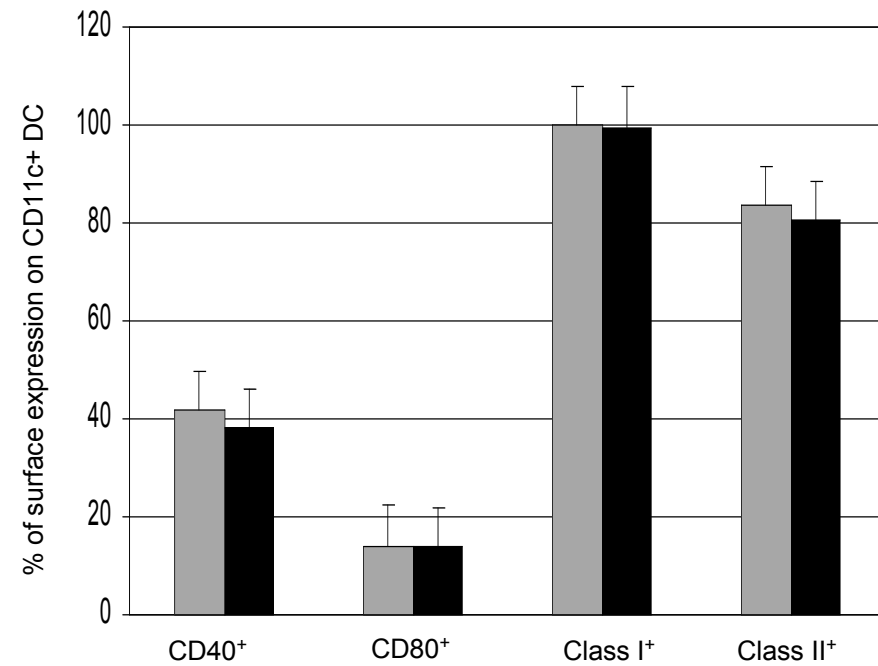


The immunosuppressive activity of mMSC is not mediated by Treg or impairment of antigen presentation

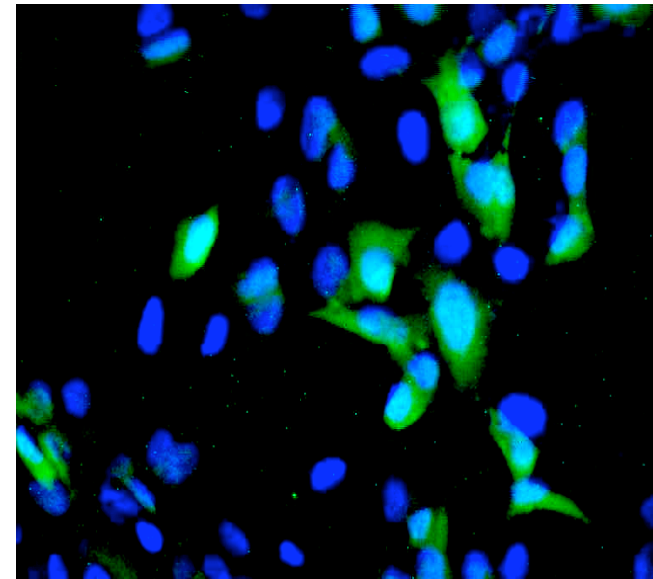
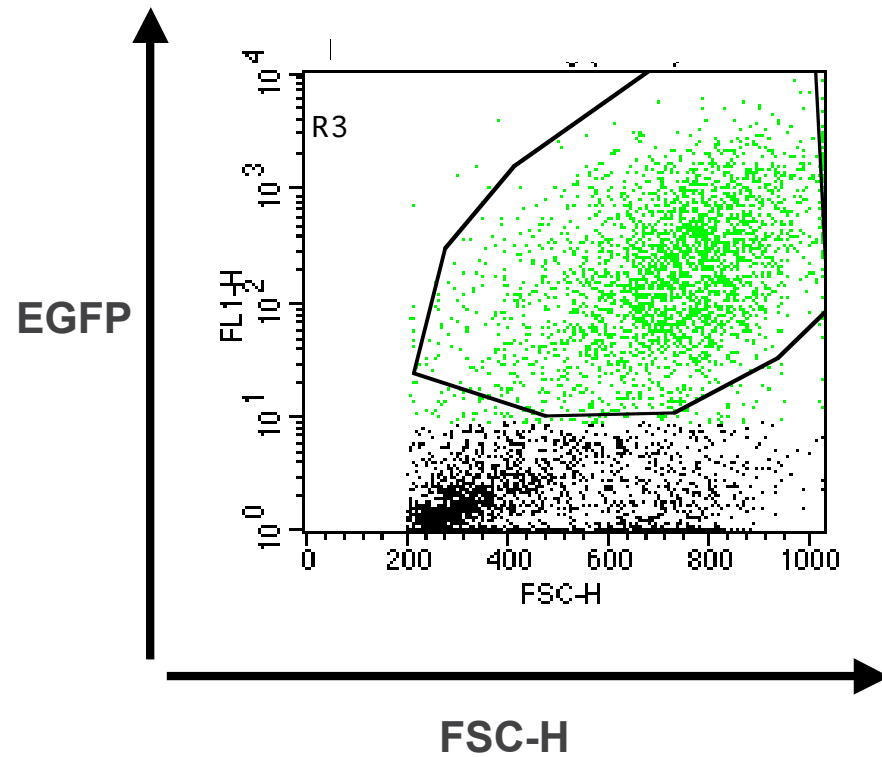
9a

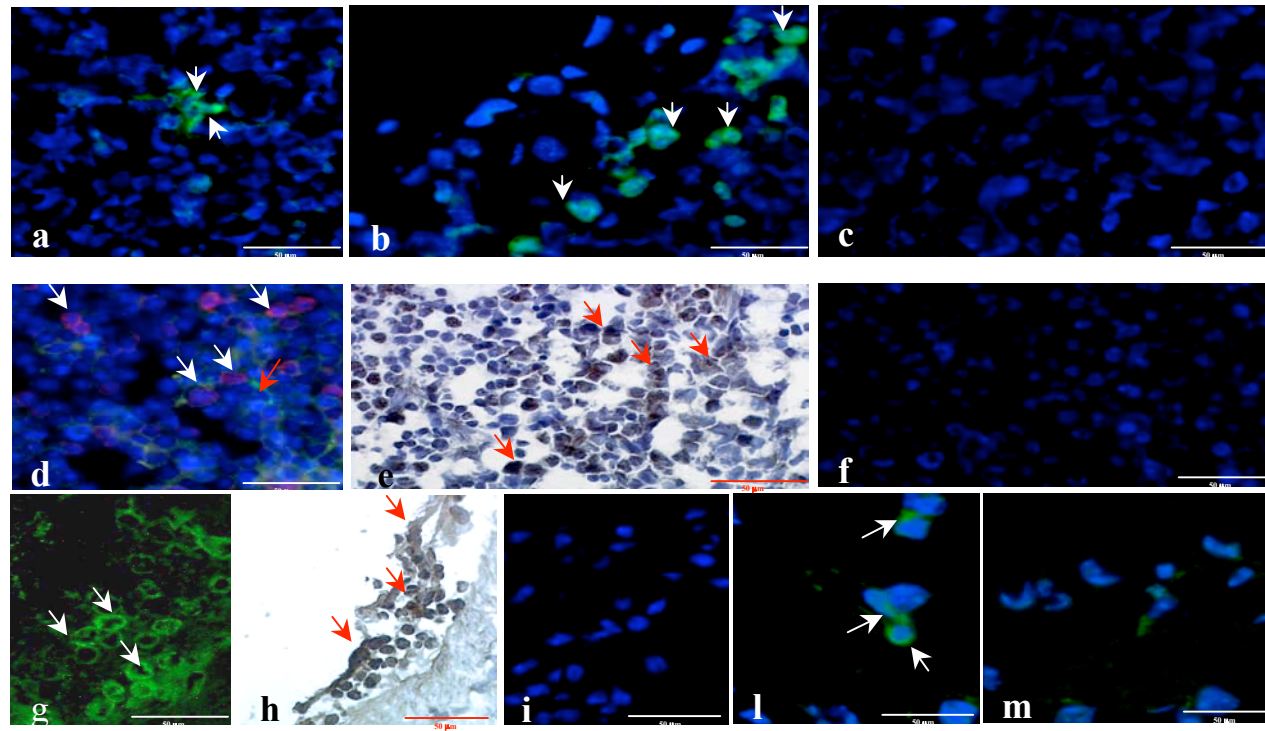


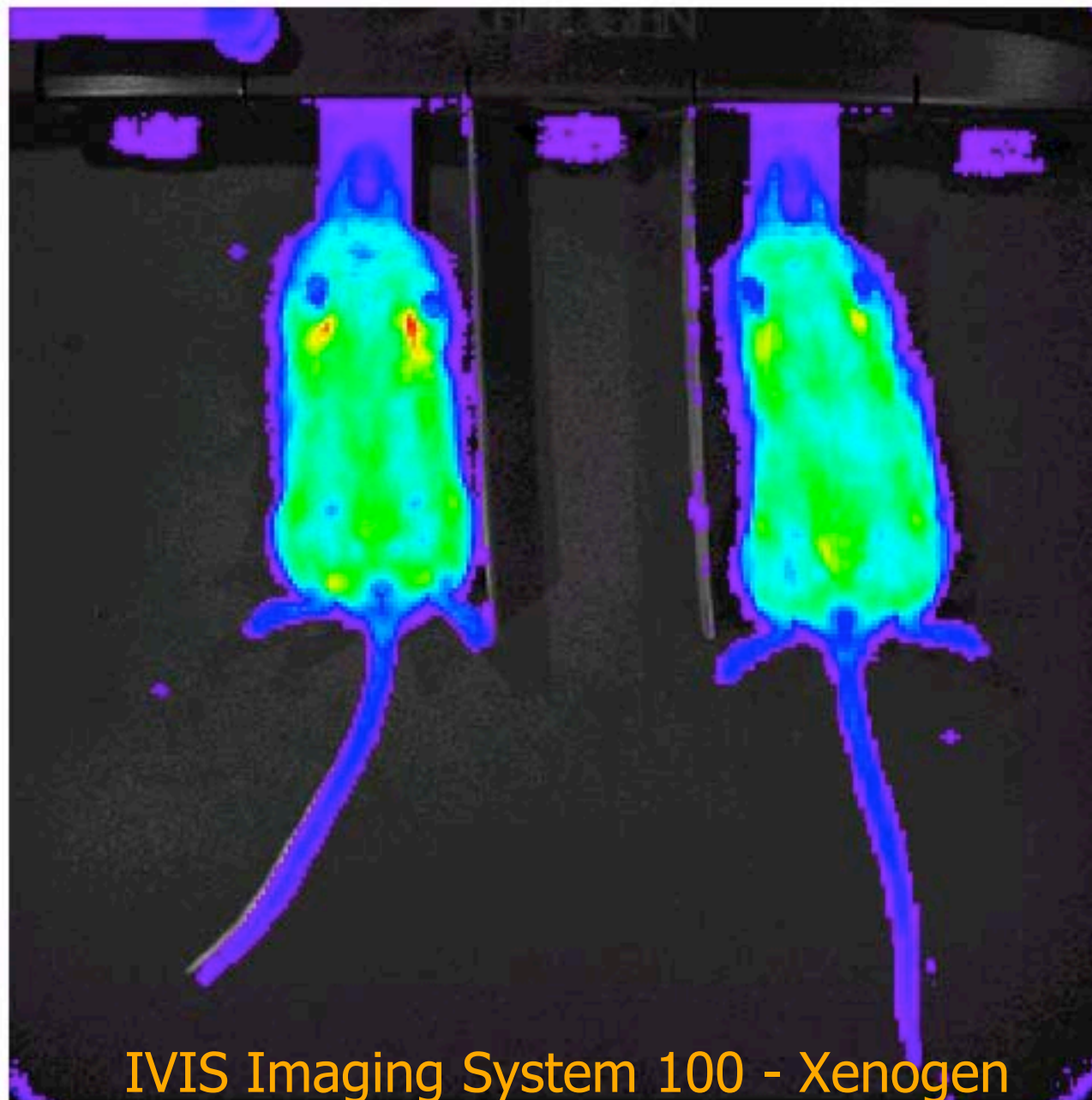
9b



mMSC transfection with the eGFP reporter protein





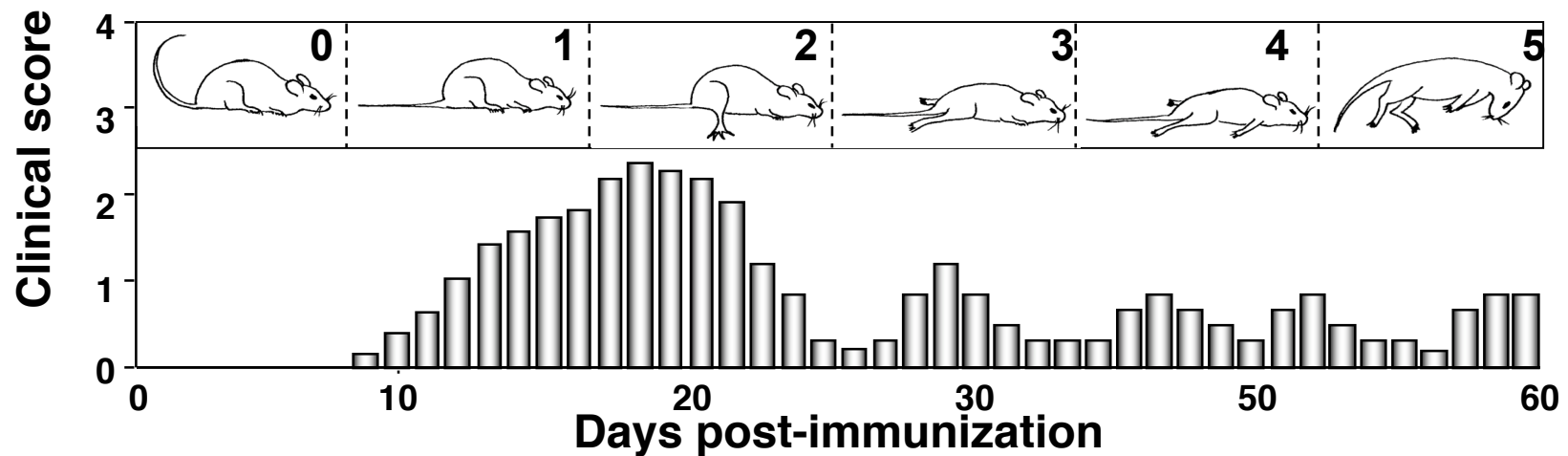


IVIS Imaging System 100 - Xenogen

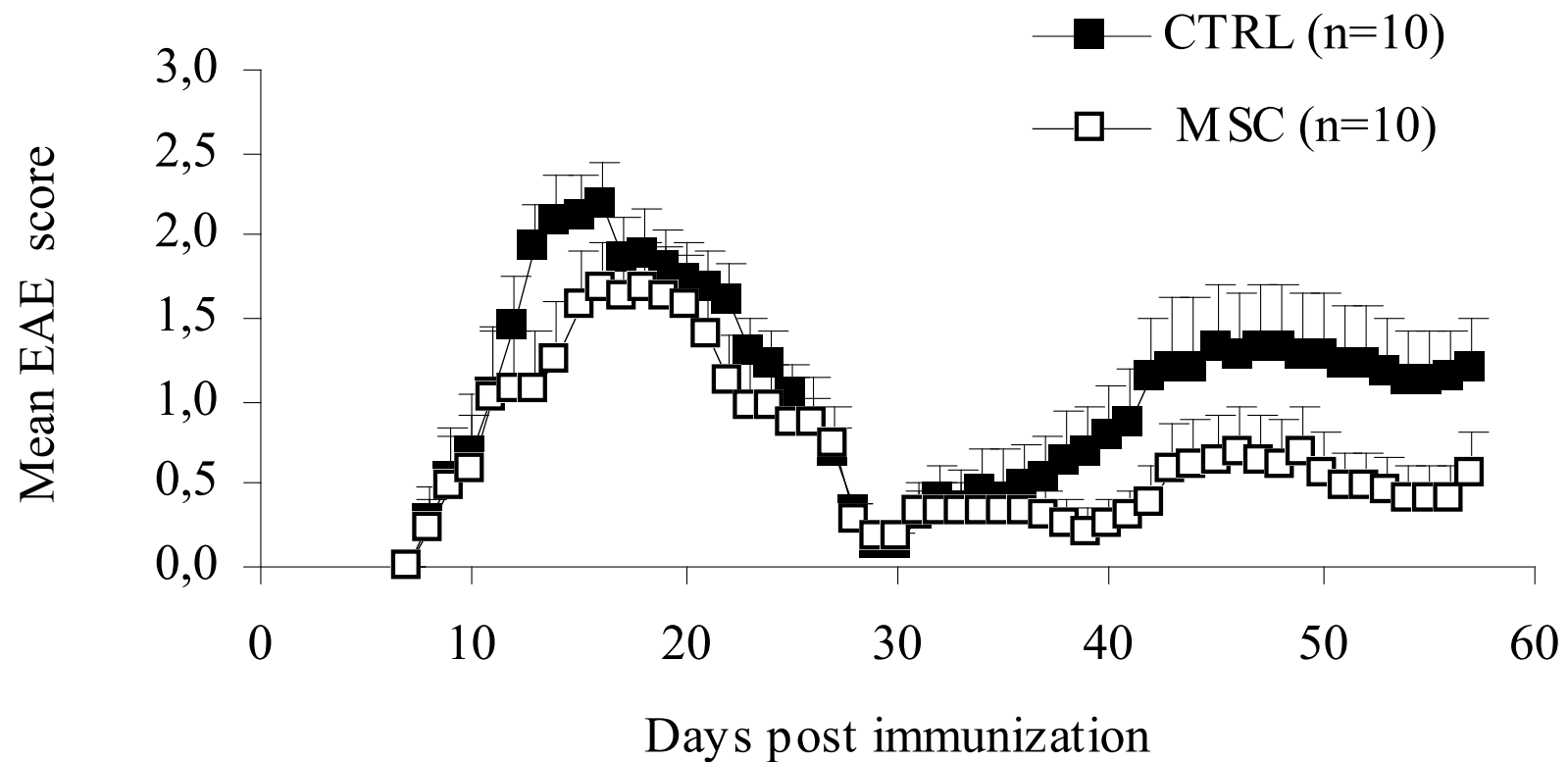
PLP139-151-induced EAE in SJL mice

Active immunization: by s.c. injection of CFA, pertussis toxin and myelin antigens

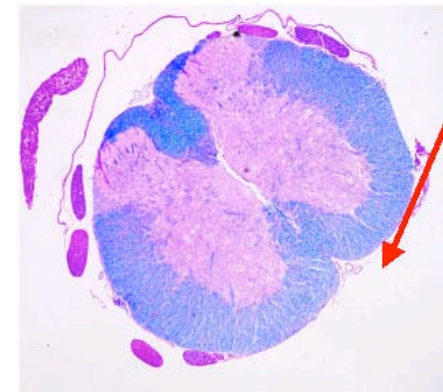
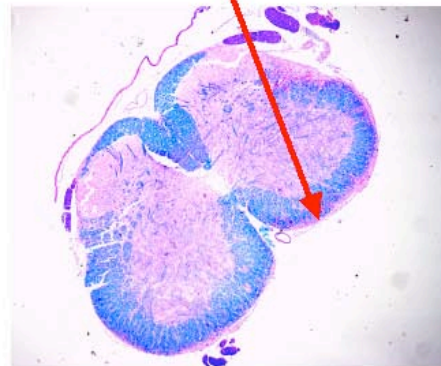
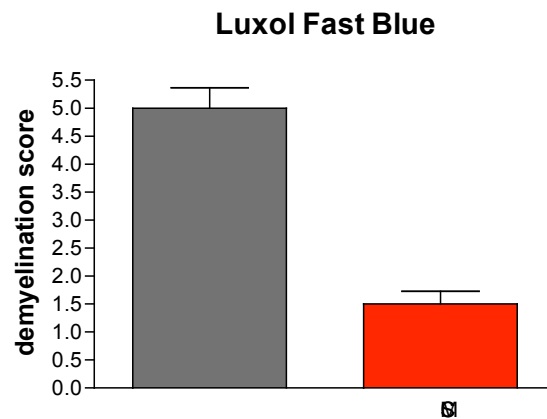
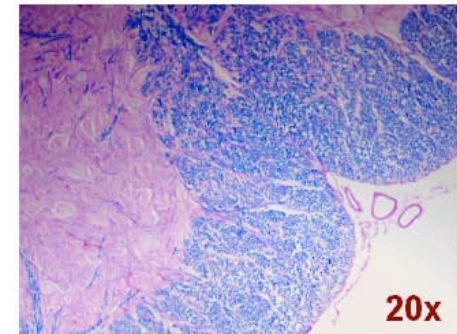
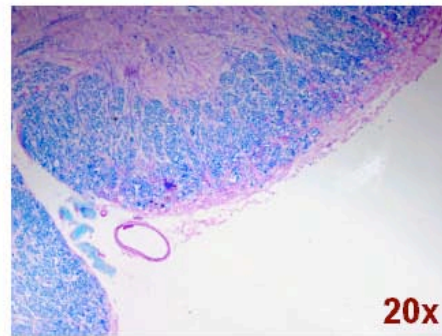
Passive transfer: by i.v. injection of activated CD4⁺-Th1 myelin-specific T cells



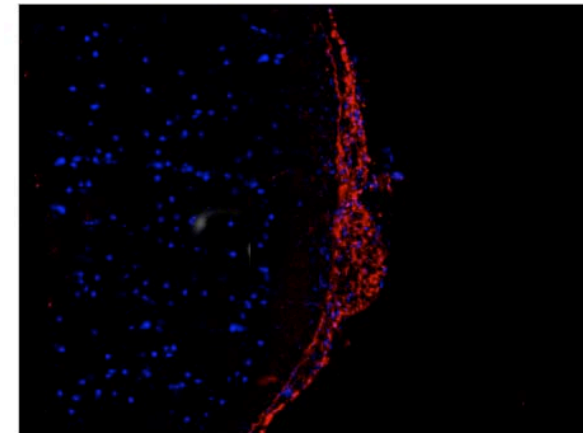
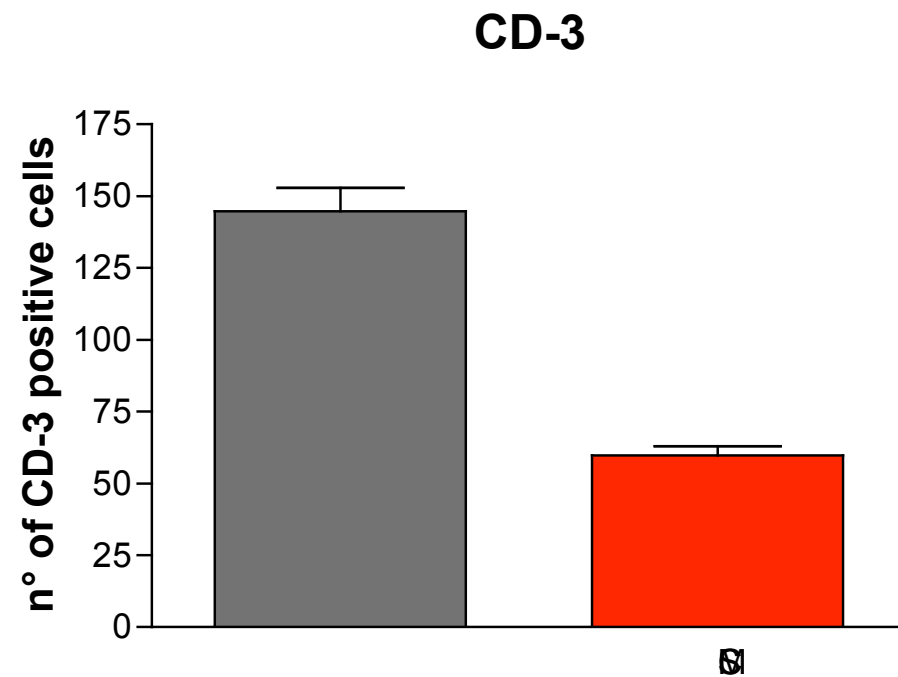
MSC inhibit relapsing remitting EAE



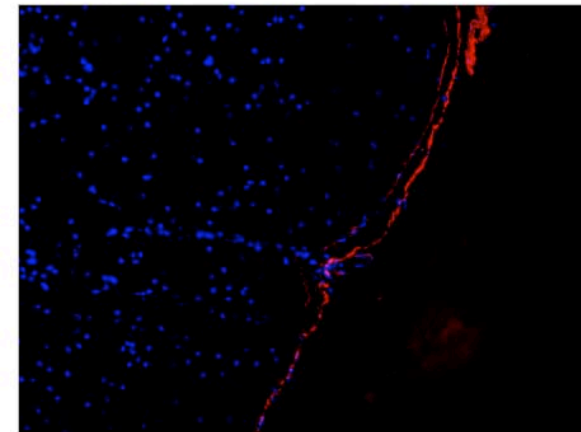
MSC inhibit subpial demyelination



MSC administration reduces CD3+ infiltrates

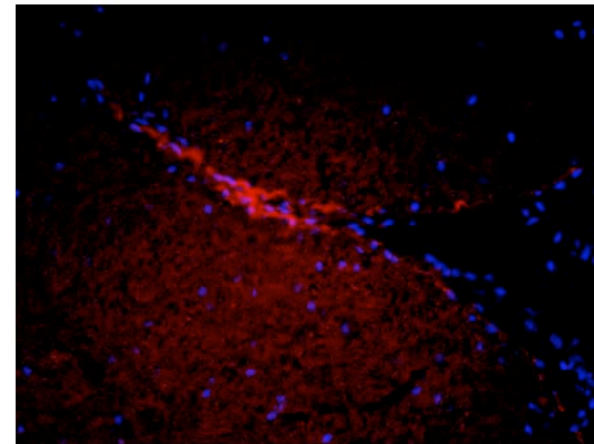
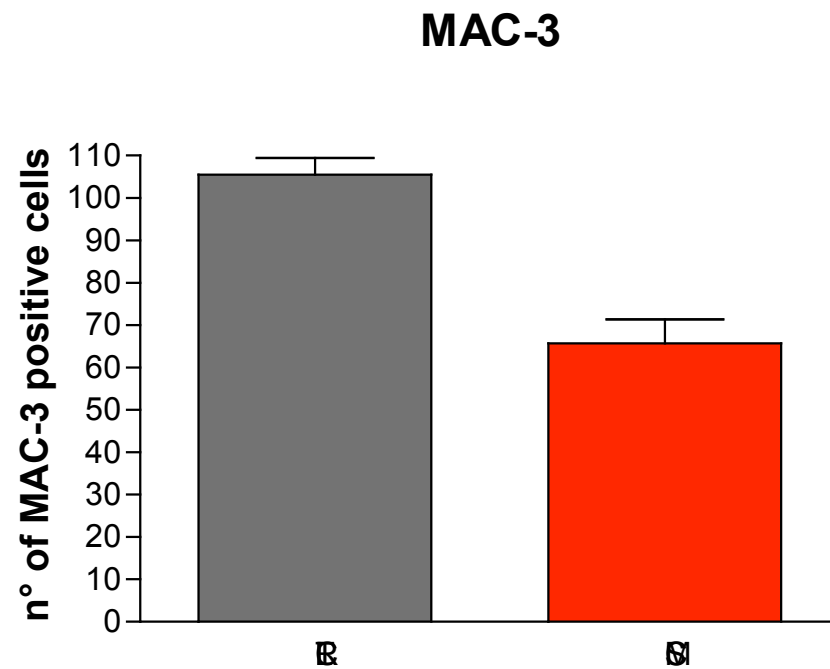


CTRL 20x

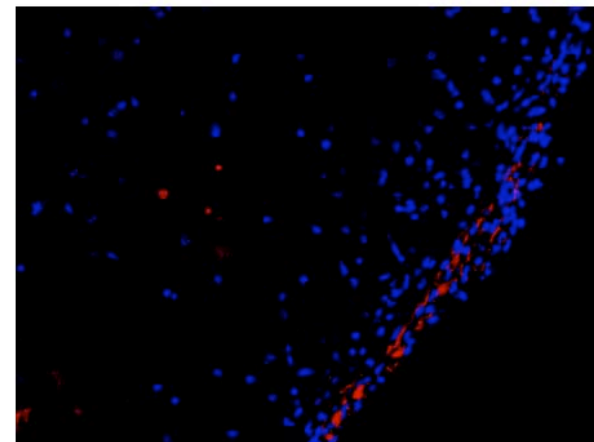


MSC 20x

MSC administration reduces MAC-3+ infiltrates

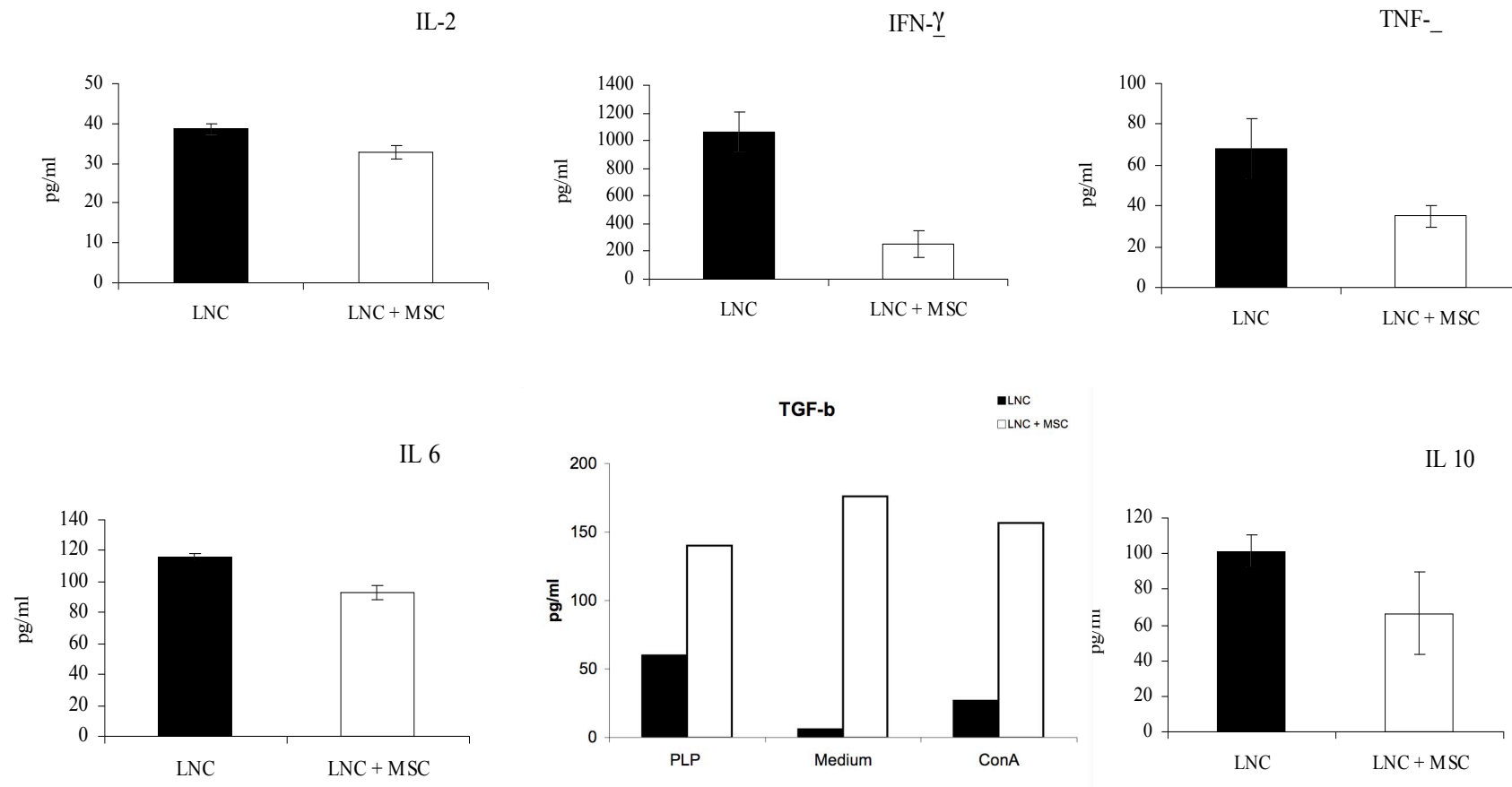


CTRL 40x

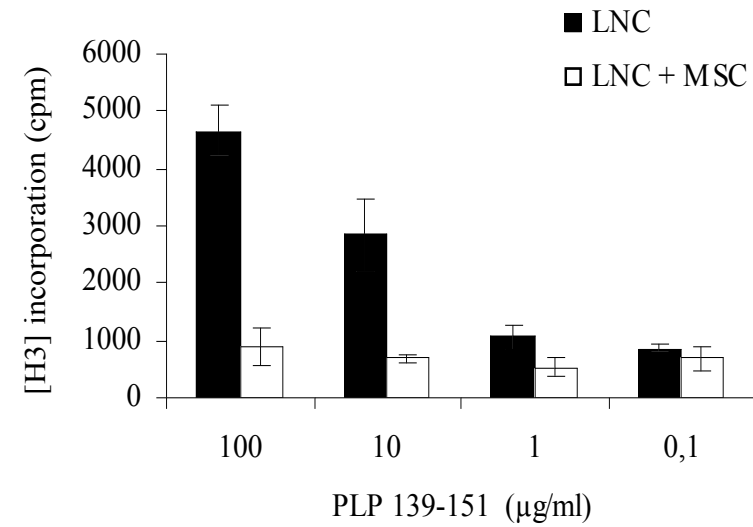
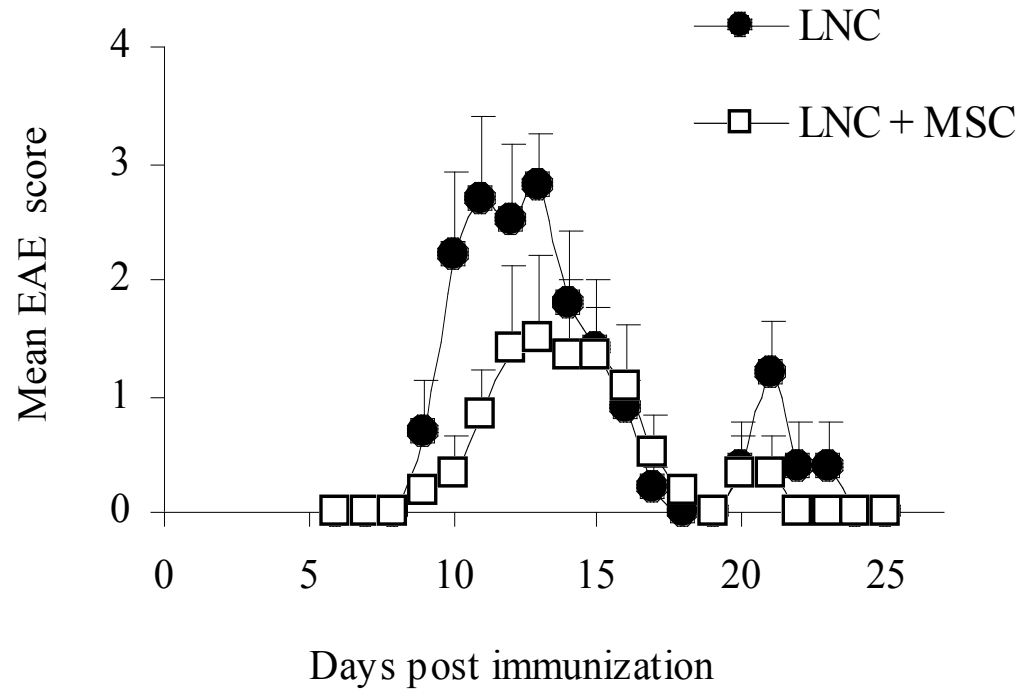


MSC 40X

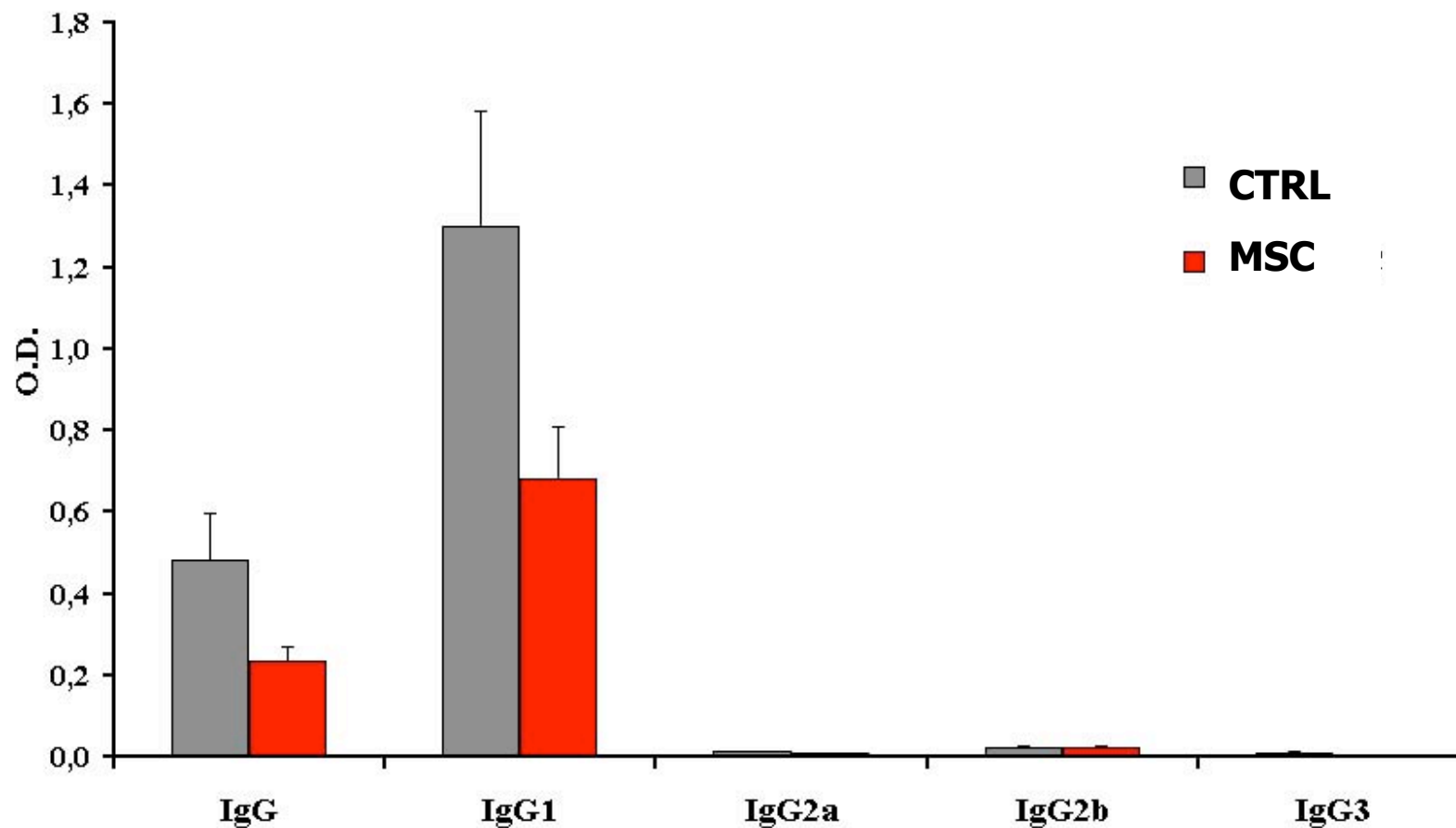
MSCs *in vitro* modulate cytokine profile of T cells activated against PLP 139-151

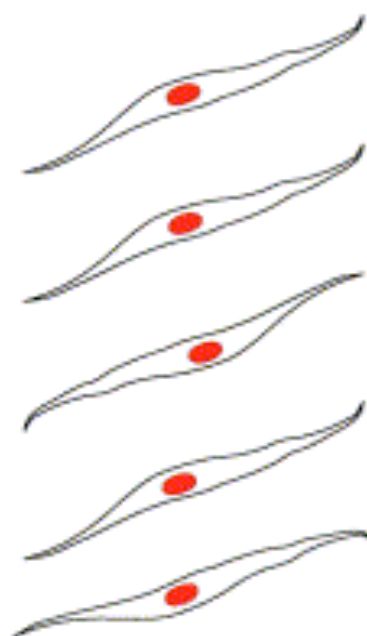
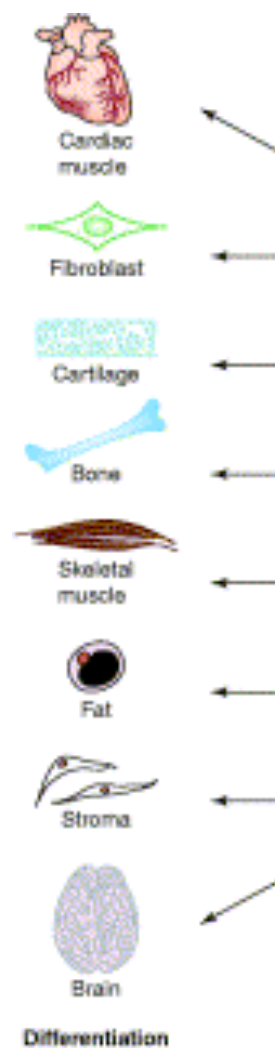


MSCs ameliorate adoptively transferred EAE



Administration of MSC inhibit in vivo IgG production

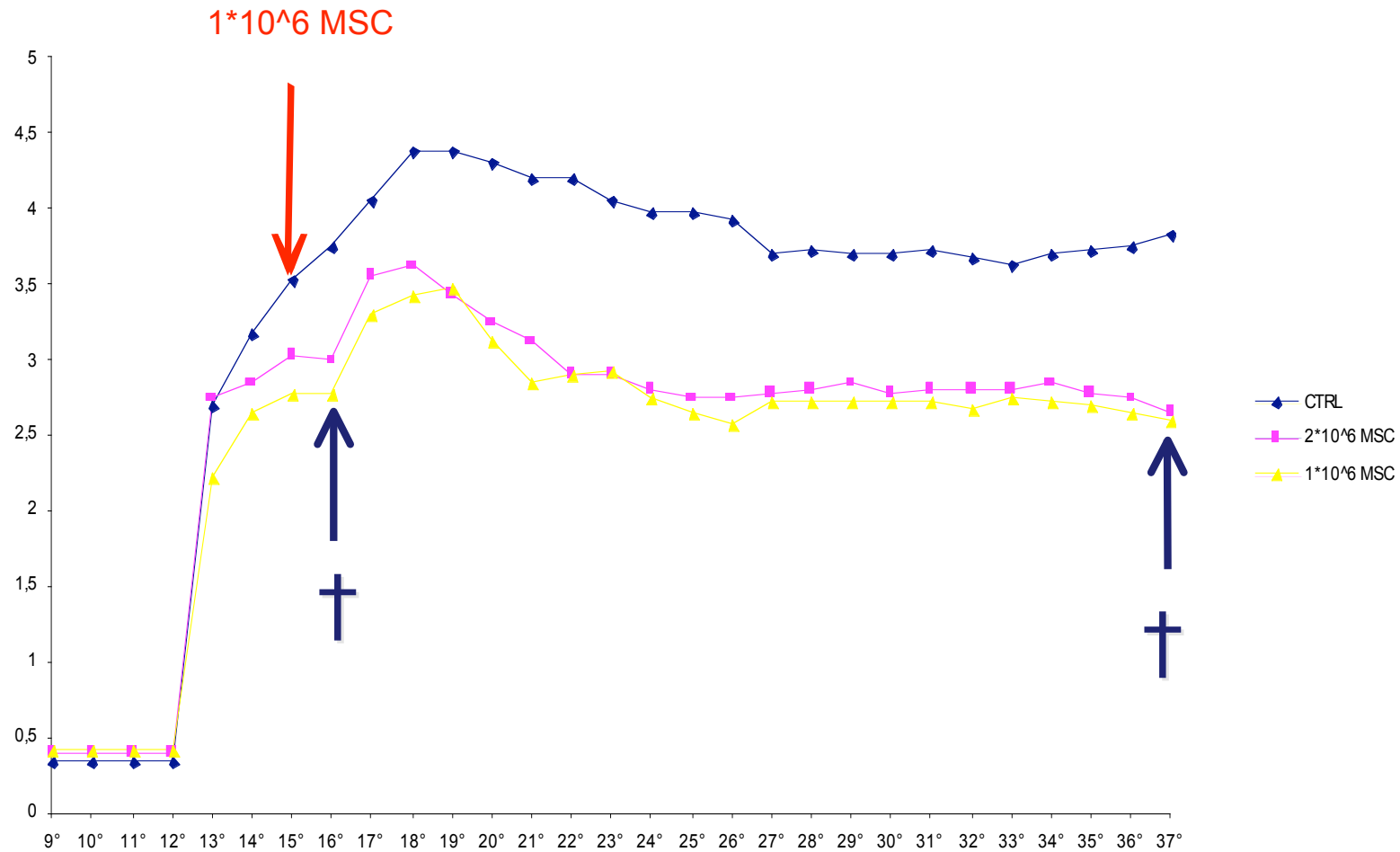




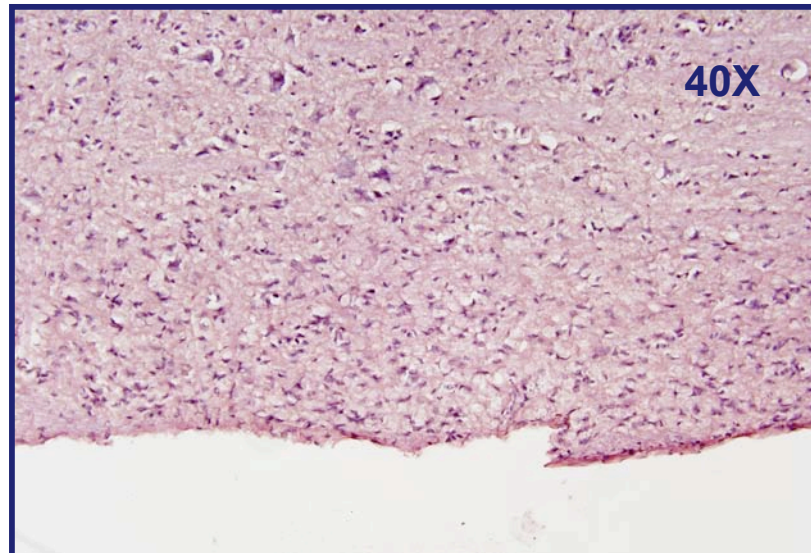
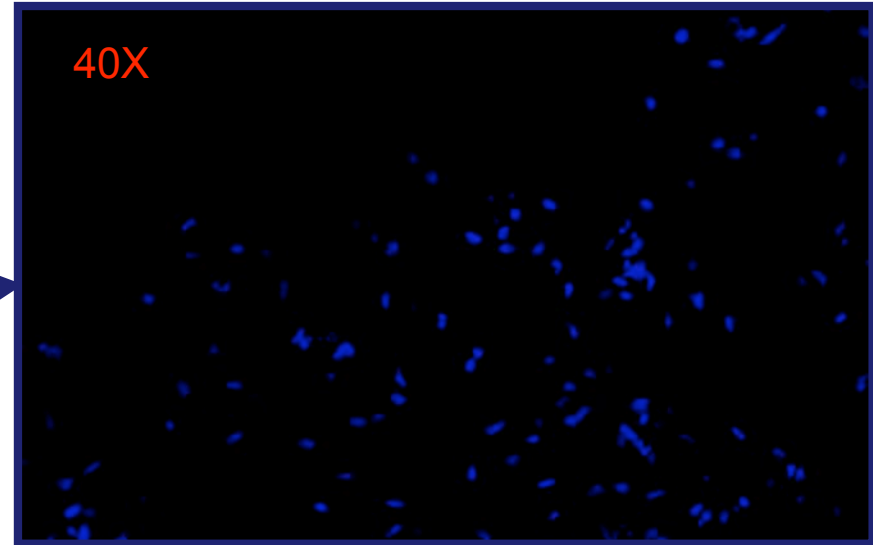
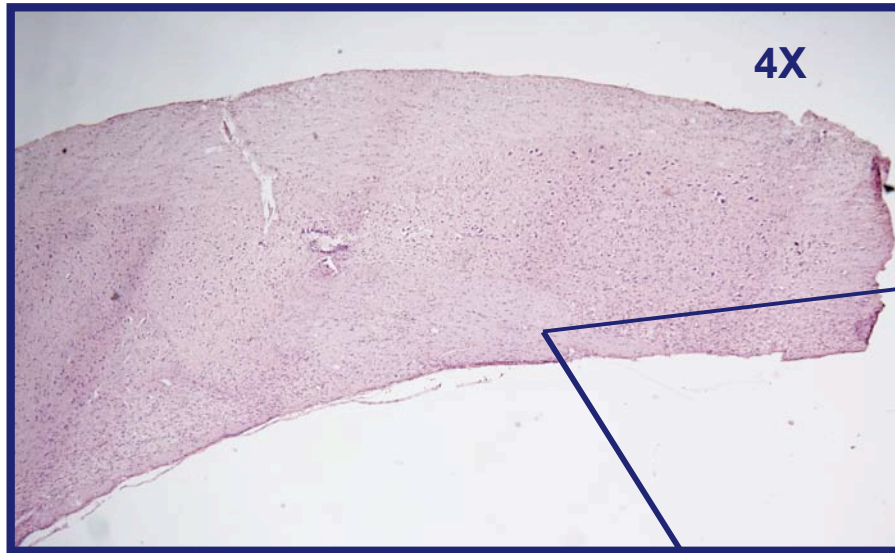
Fusion

TRENDS in Biotechnology

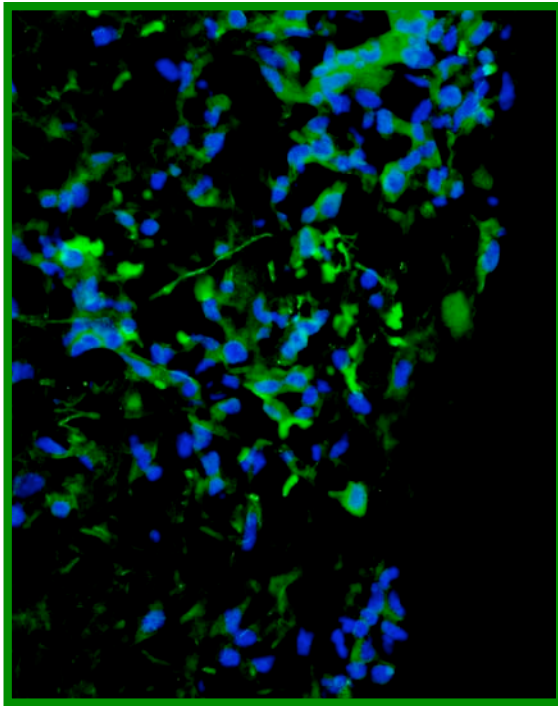
Homing experiments



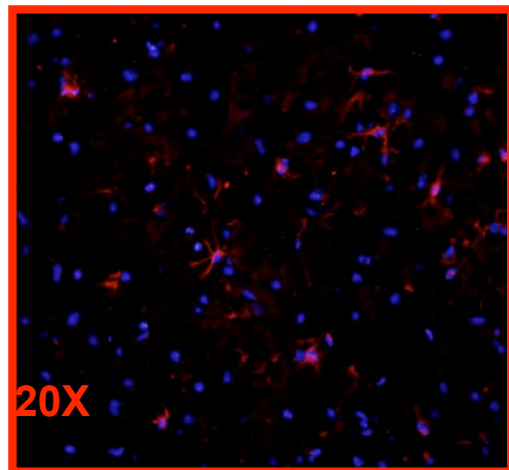
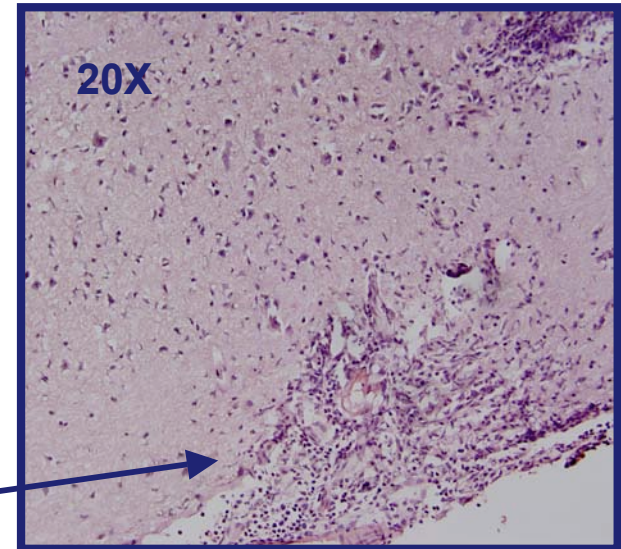
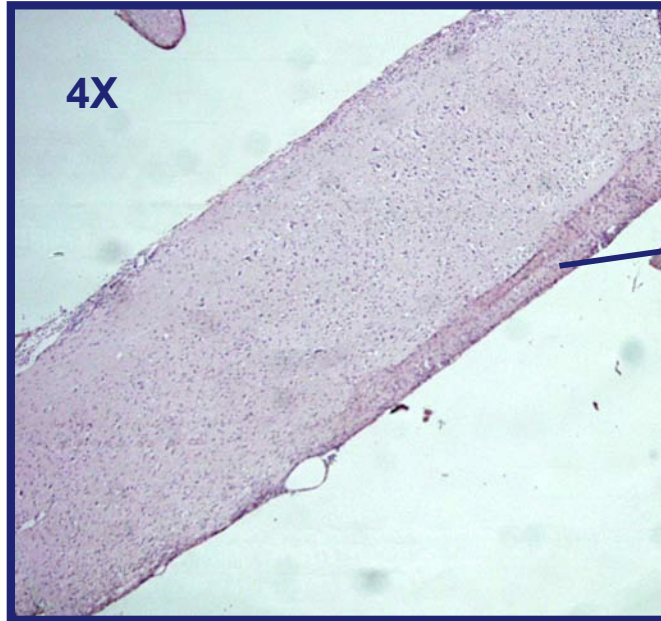
@ 24 hours



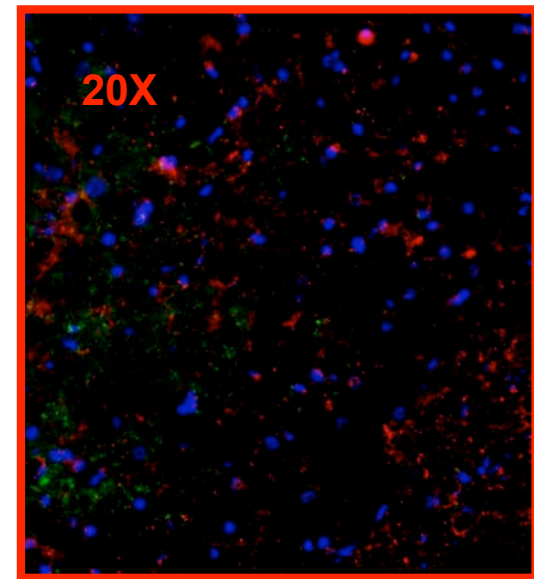
@ 30 days



GFP+(G),DAPI(B)

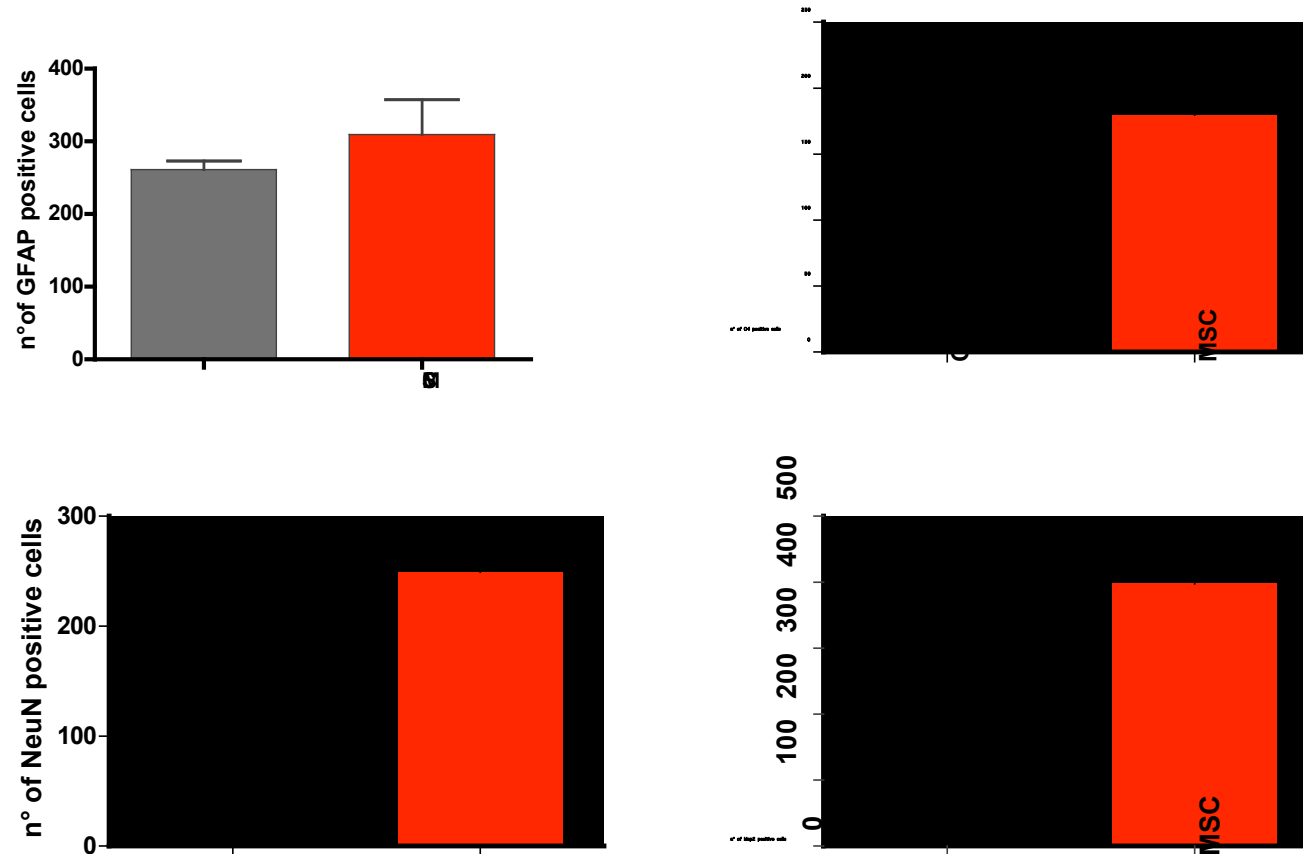


GFAP+ (R), DAPI (B)

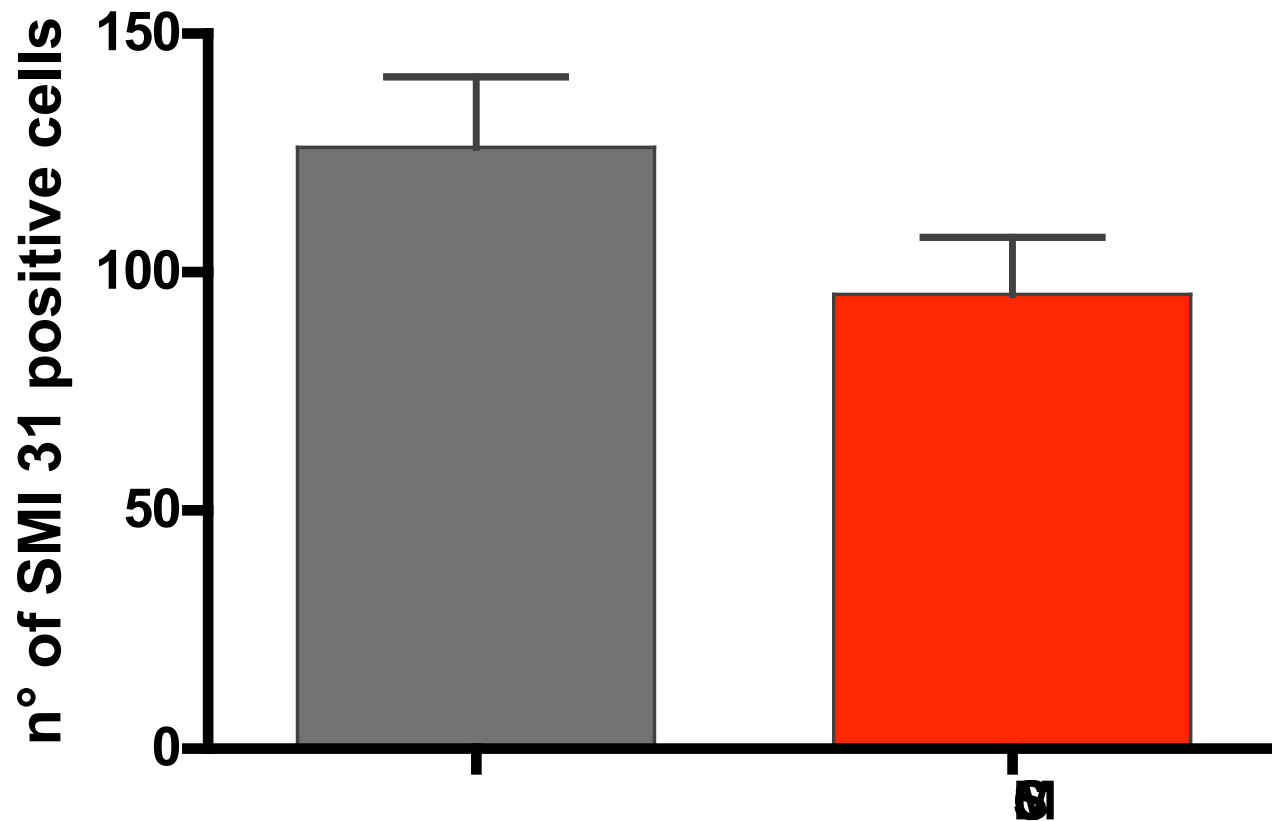


GFP+ (G), NeuN (R), DAPI (B)

Neural cells in MSC treated and control mice

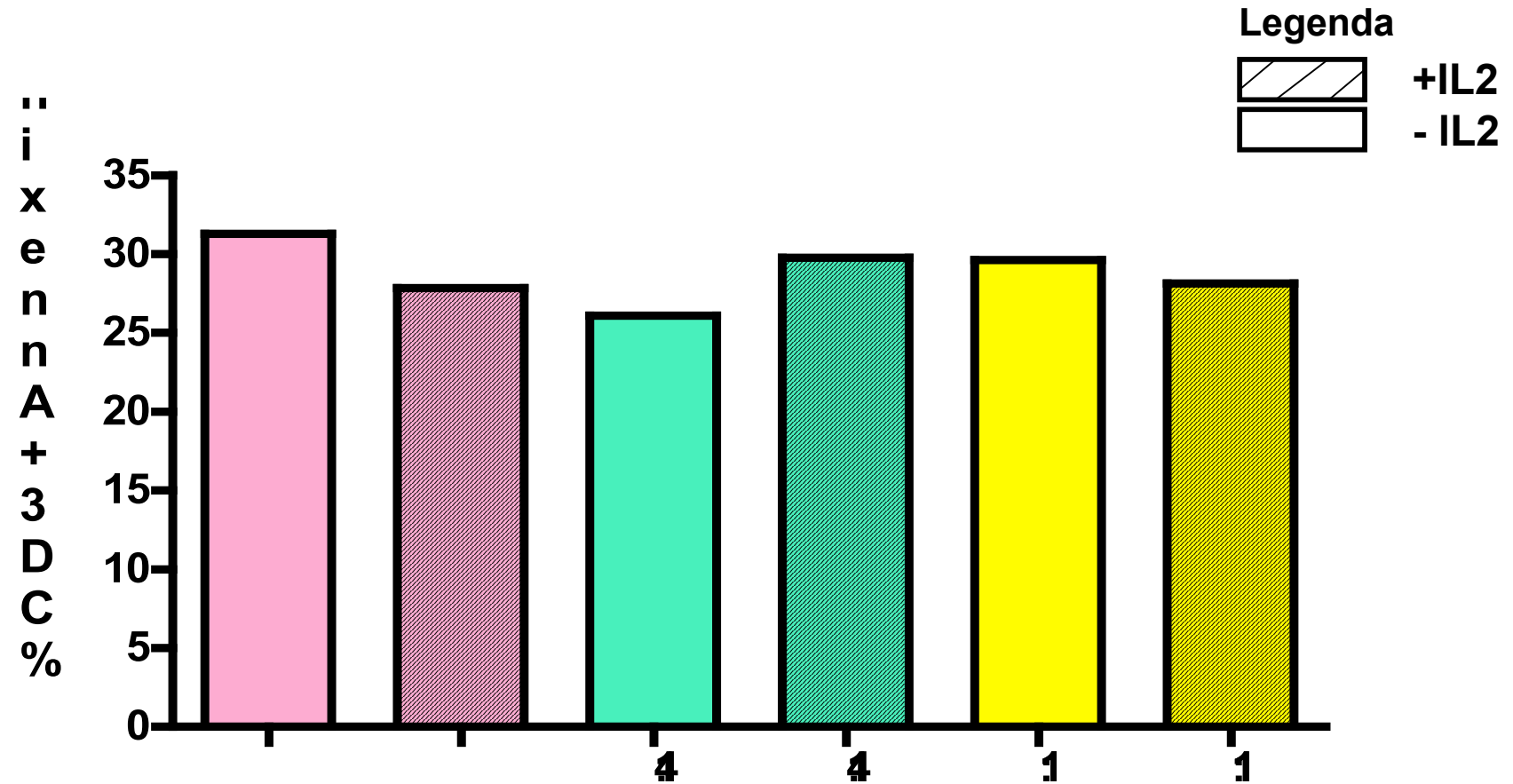


MSC decrease axonal sufferance

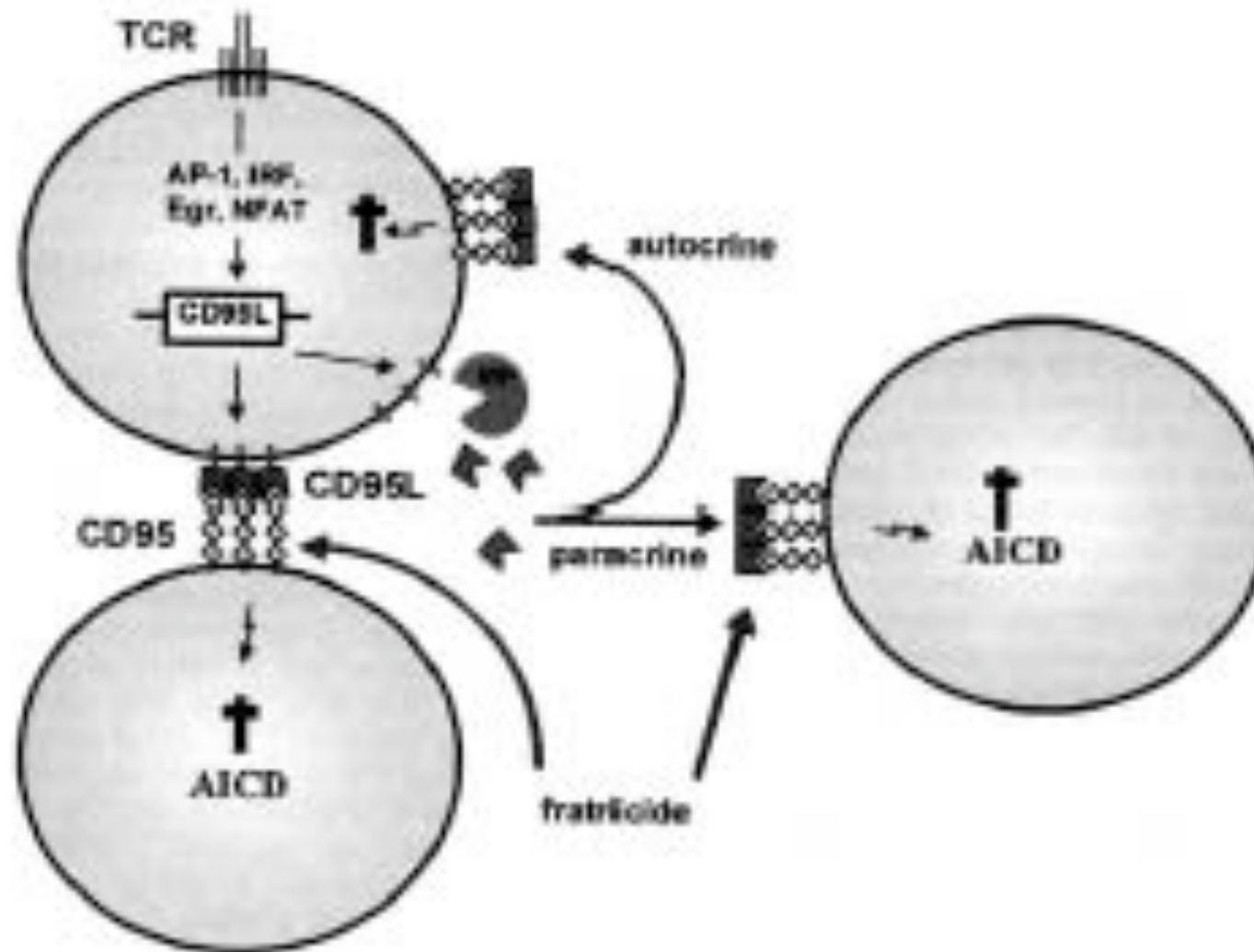


MSC & apoptosis

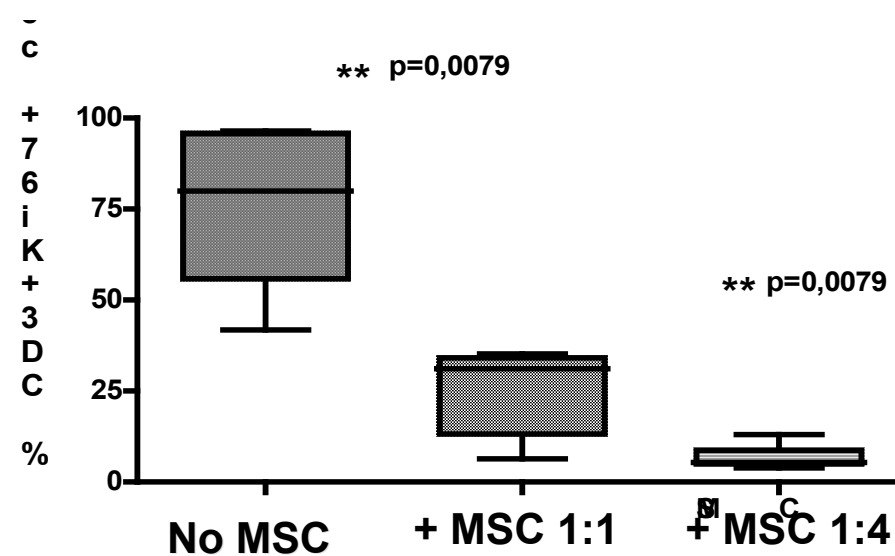
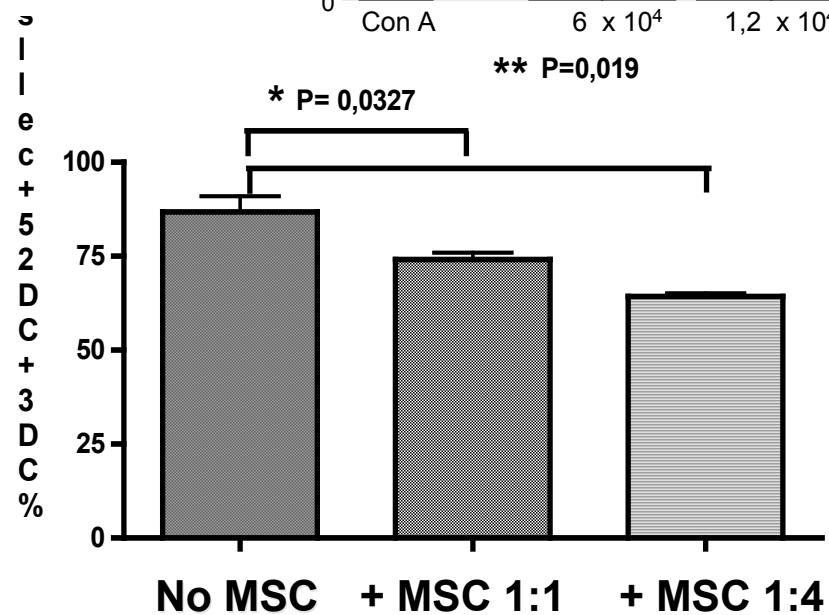
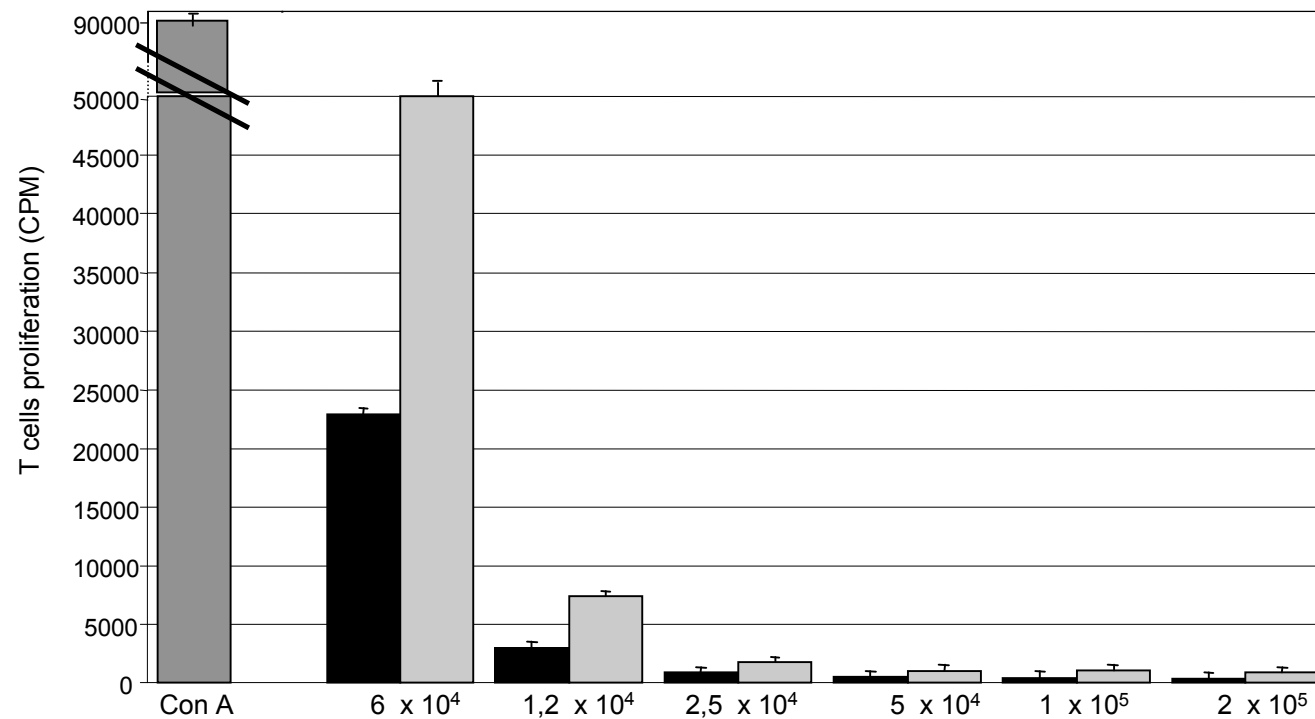
The inhibition of PBMC proliferation is NOT due to apoptotic mechanisms



Model for Activation Induced cell-death

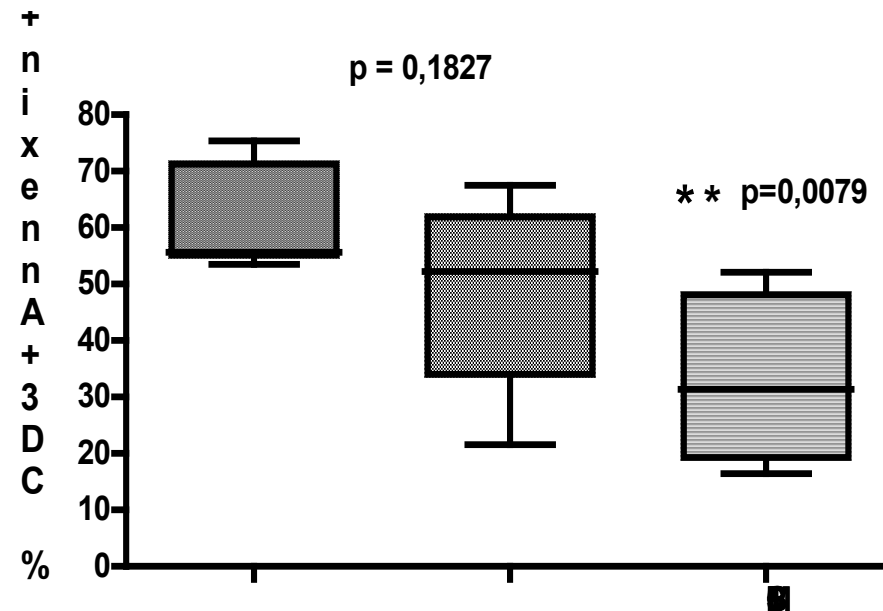


[Baumann et al. Current Molecular Medicine 2002; vol. 2:257]

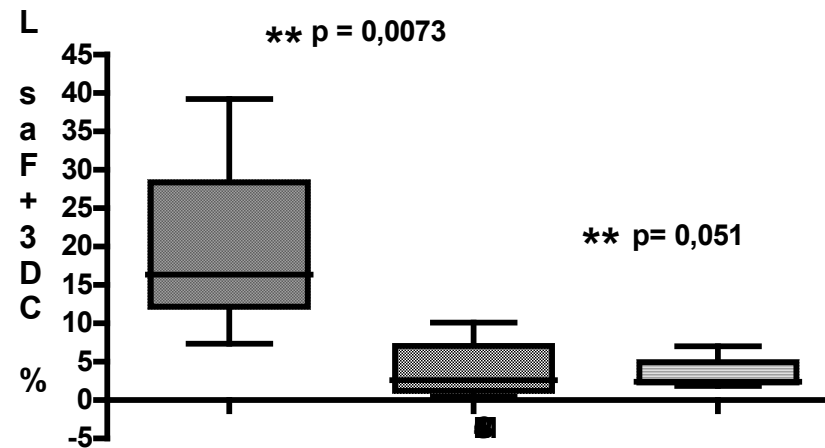
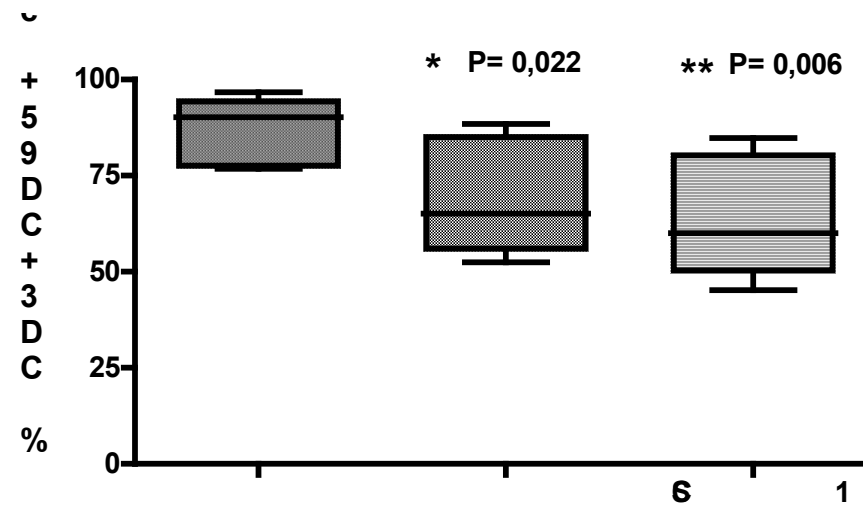


AICD Protocol

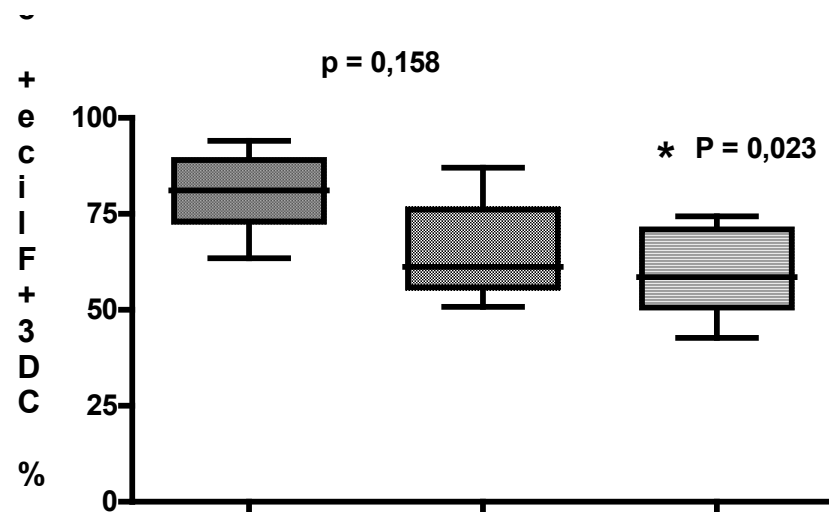
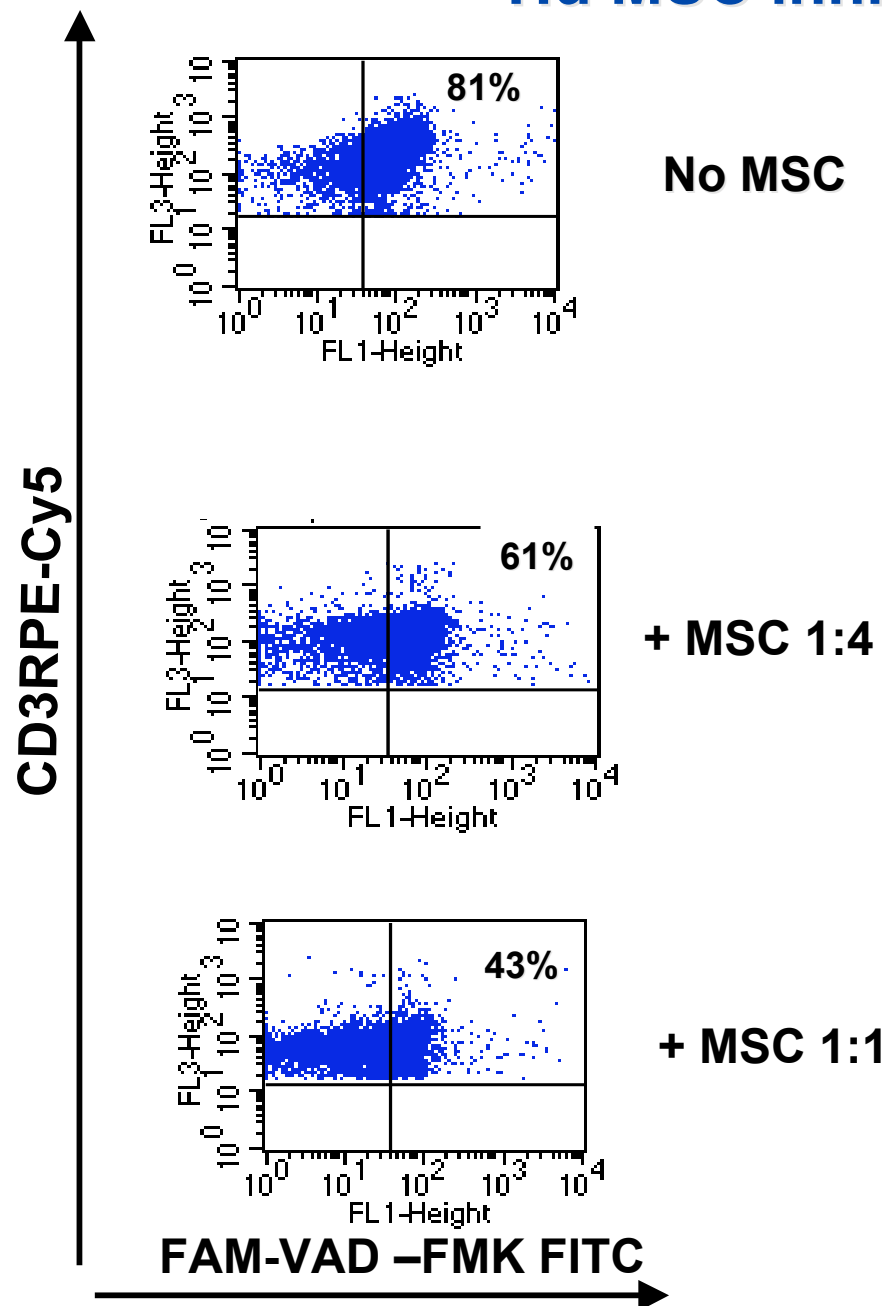
MSC decrease the frequency of CD3+Annexin + cells and...



... downregulate Fas e Fas L on CD3+ cells

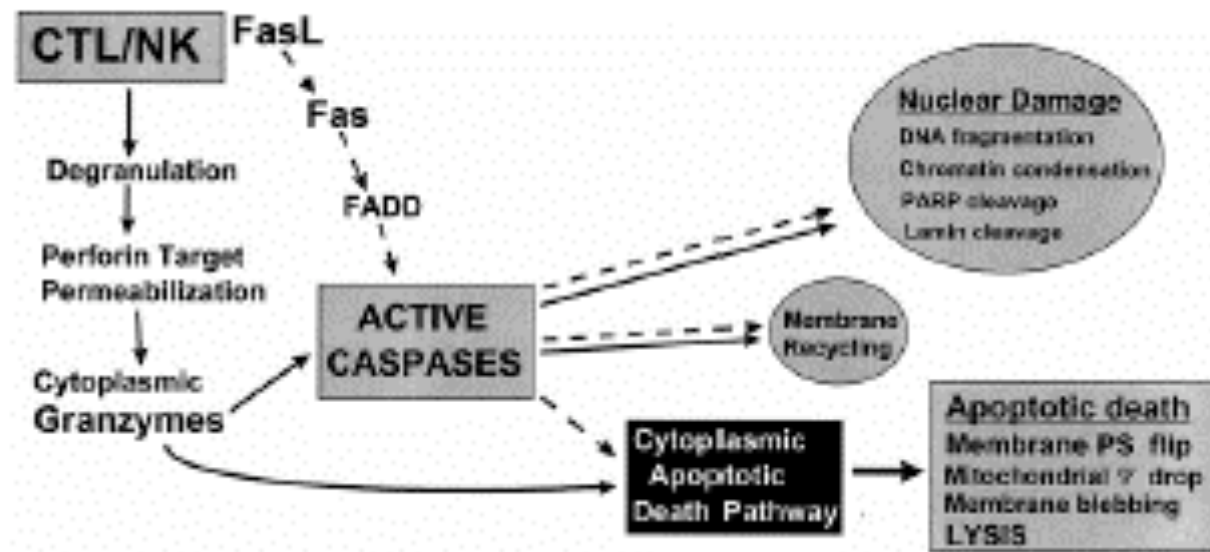


Hu MSC inhibit activation of total caspases

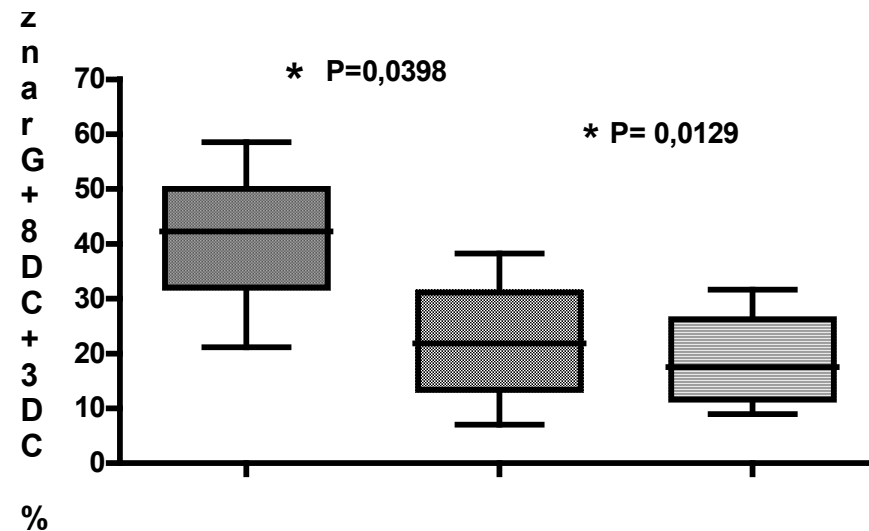
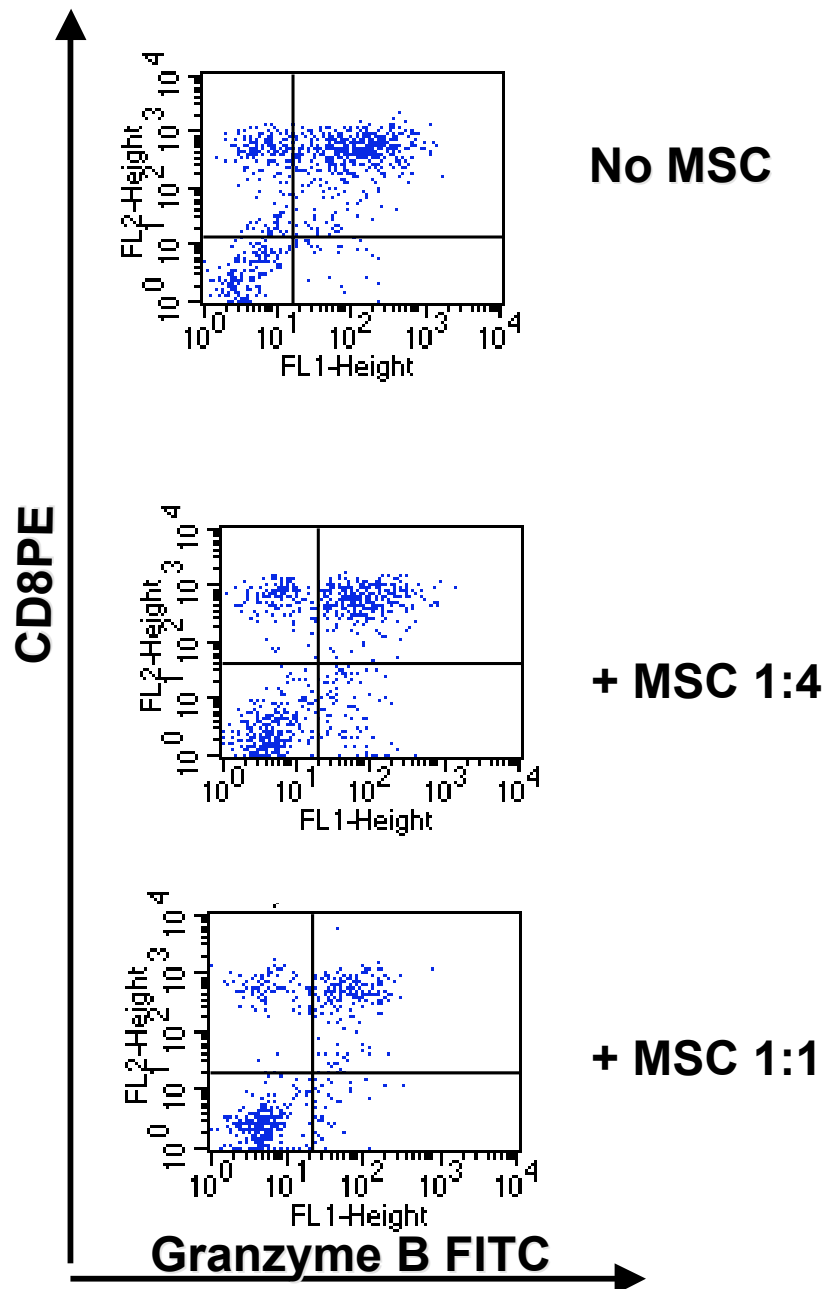


Granzyme B

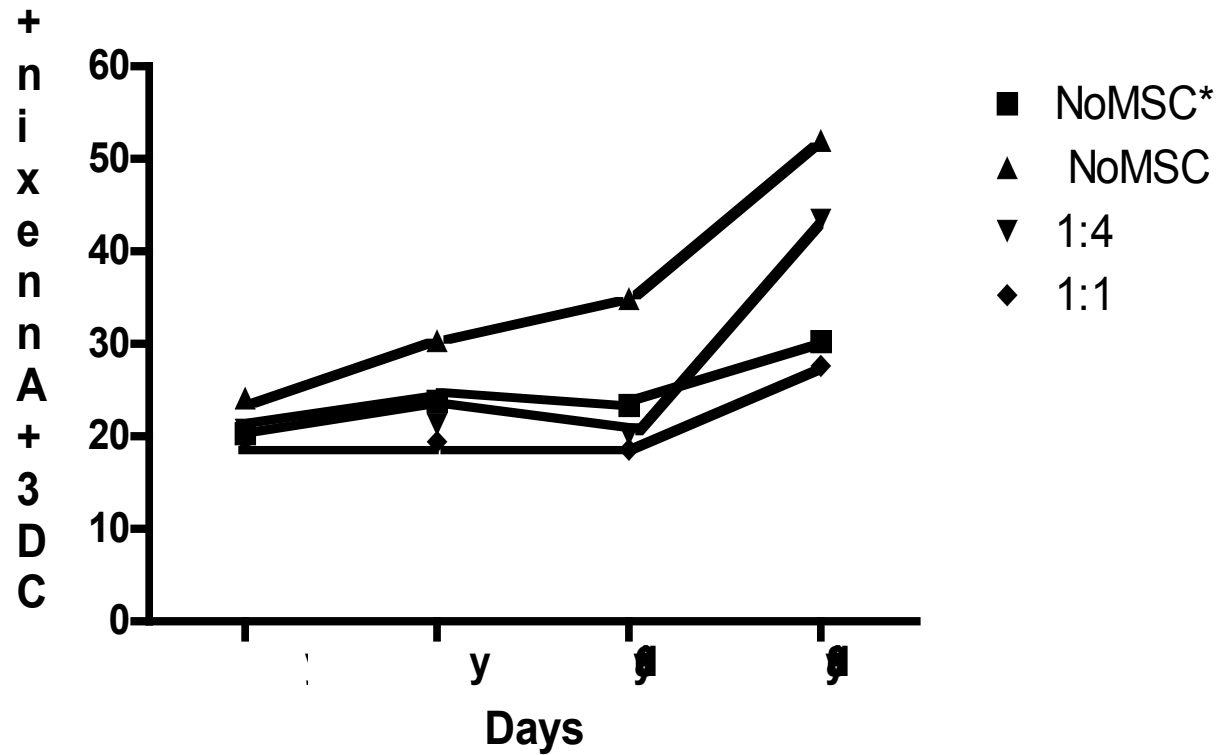
- It is a serine protease stored in the granules of cytotoxic T lymphocytes and NK cells along the pore-forming protein Perforin
- In cell-mediated responses, Granzyme B is directly involved in target cell-lysis
- It acts on several target substrates in the nucleus and in the cytoplasm including specific caspases (-3,-7,-9,-10), cleaving them following Asp residues



HuMSC also decrease Granzyme B+ of CD3+CD8+cells



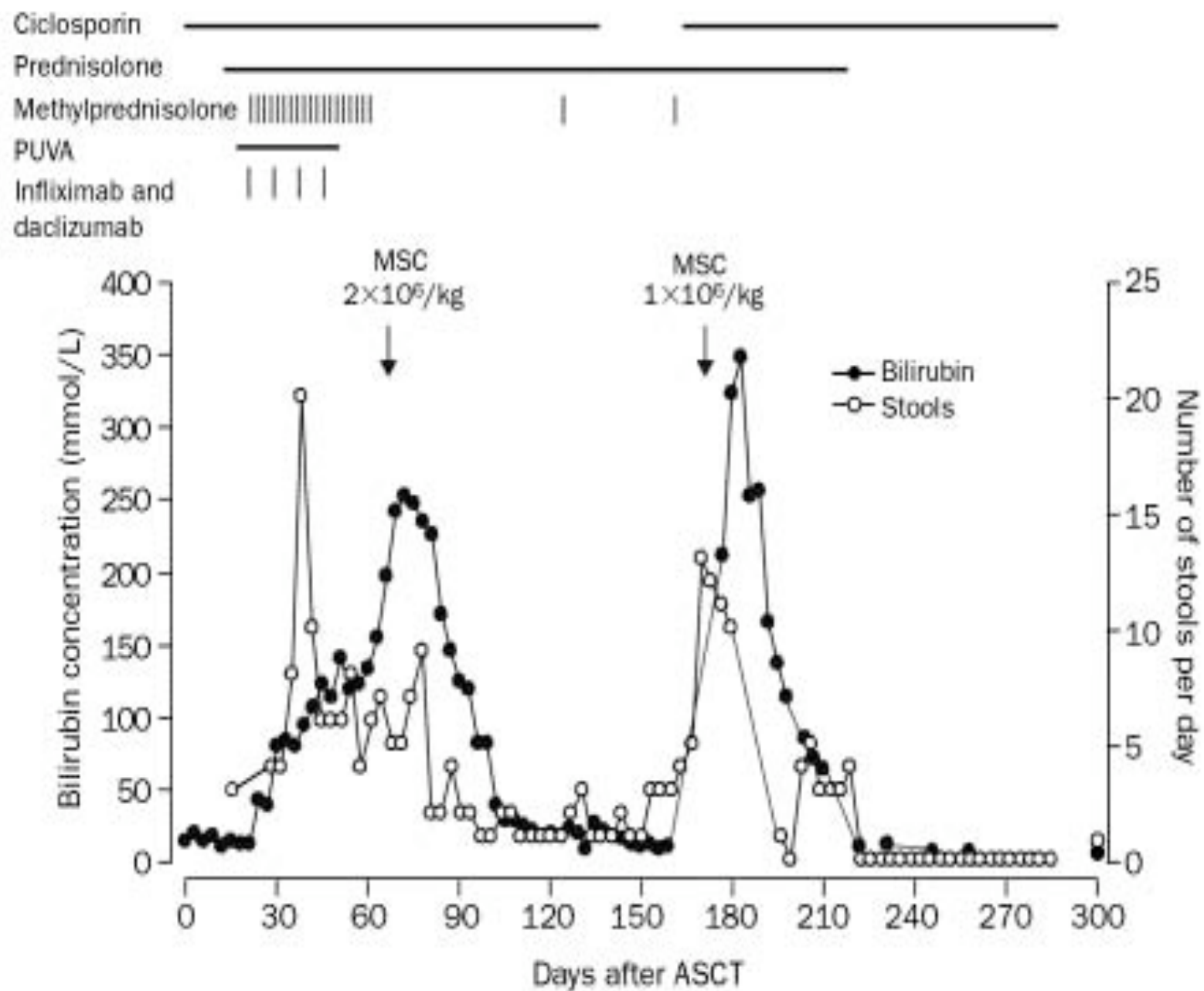
MSC support PBMC survival



No MSC* = FCS-enriched medium
 NoMSC = Serum-free medium

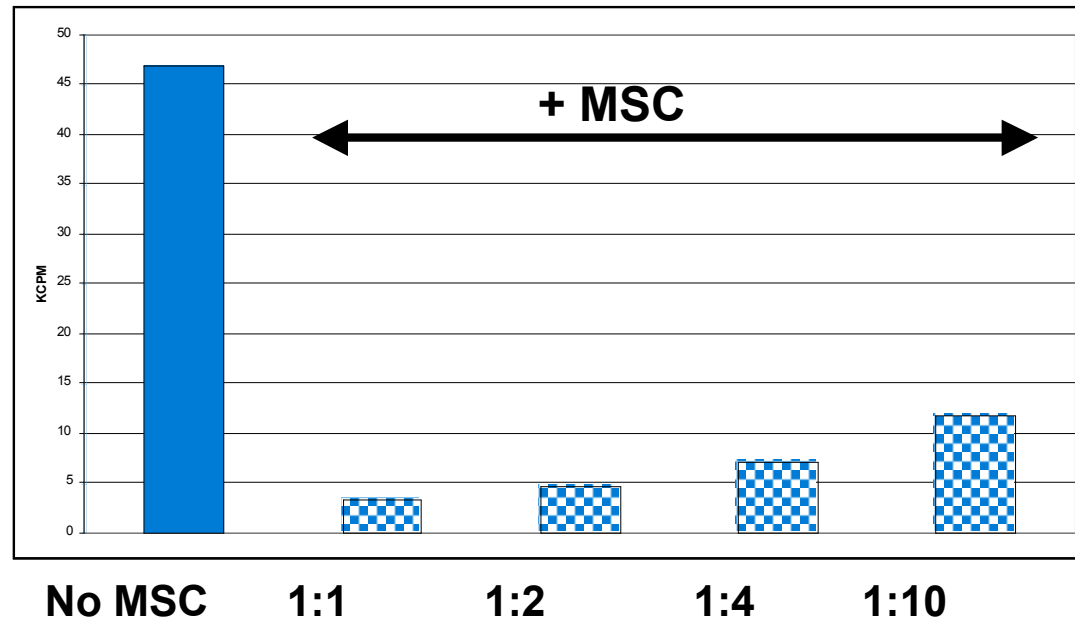
Conclusions

- These data suggest that the immunoregulatory properties of MSC effectively interfere with the autoimmune attack in the course of EAE inducing an in vivo state of T cell unresponsiveness occurring within secondary lymphoid organs
- MSC ameliorate also PLP induced EAE through a T cell mediated mechanism that could possibly result also in an impairment of B cell responses.
- Upon IV injection, MSC early engraft inside the lymphoid organs and, at later stage, reach the subarachnoid space and then diffuse inside the inflamed parenchyma
- MSC rescue T cells from AICD
- The combined effect of MSC on T and B lymphocytes set up the stage for the treatment of autoimmune demyelinating diseases such as MS (Frank M, Sayegh M. Lancet 2004, Burt R, Blood 2005)

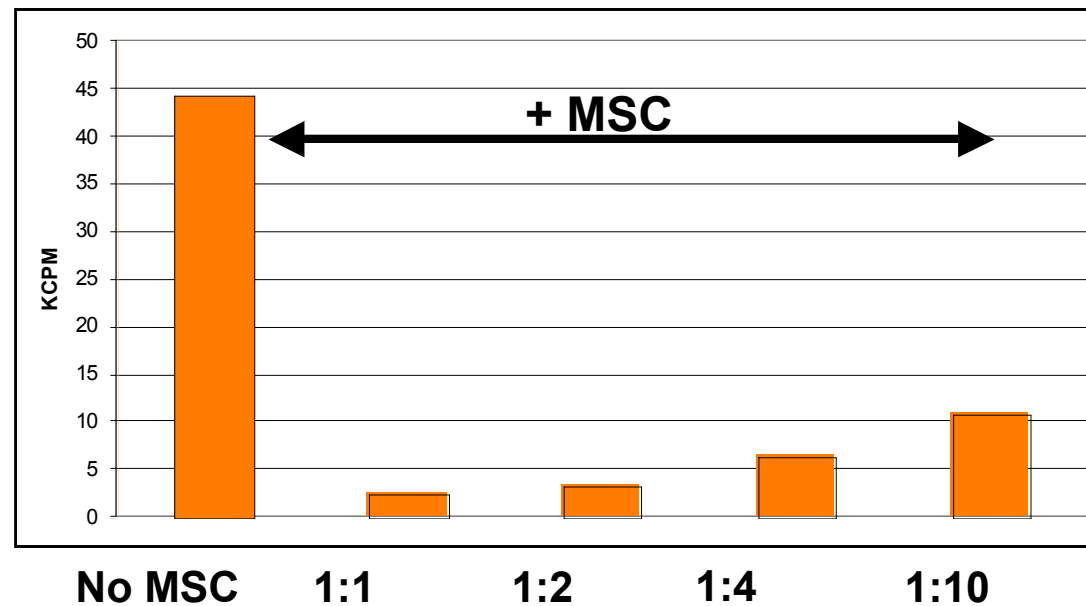


MSC from MS donors inhibit anti CD3+CD28 induced T cell proliferation

MSC from healthy donors



MSC from MS patients



Benvenuto et al, personal communication

ACKNOWLEDGEMENTS

- **Neuroimmunology Unit**

- F. Benvenuto
- I. Bonanni
- S. Casazza
- F. Cazzanti
- A. Ceravolo
- S. Chiesa
- S. Ferrari
- E. Gerdoni
- D. Giunti
- E. Pedemonte
- A. Uccelli

- **Dept. Of Neurosciences**

- E. Capello
- GL. Mancardi

