CCR2 dependent predictive value of circulating monocyte number on mouse survival is lost after cyclophosphamide treatment in a EL4-metastatic mouse model

M. Rodero, F. Licata, P. Hamon, L. Poupel, C. Combadière and A.Boissonnas

Inserm UMR-S 945, Paris

INTRODUCTION

•Tumor Associated Macrophages (TAM) are key in tumor development outcome.

•Cyclophosphamide (CTX) is an alkylating myeloablative agent targeting cycling cells.

•The Role of chemokines in myeloid cell recovery and tumor outcome after CTX treatment is not well defined.

Aim 1: Characterization of myeloid cells repopulation kinetic in organs after CTX treatment .

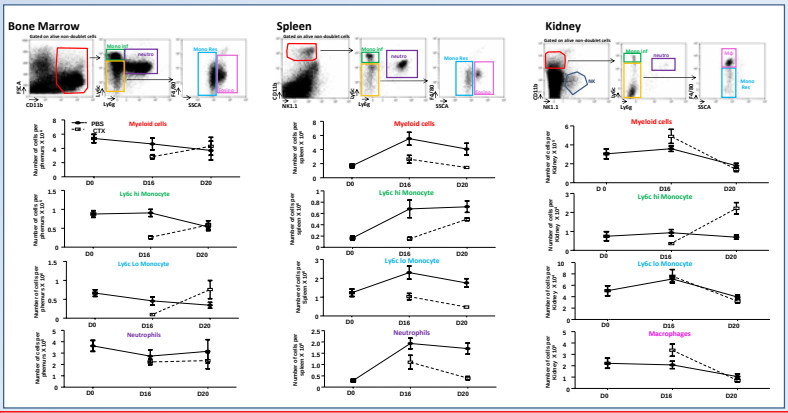
METHOD

•8-12 weeks old WT mice were injected iv with EL4 tumor cells and treated with CTX (175 mg/kg) at D15.

•Spleen, bone marrow and kidney were harvested at D16 and D20 post CTX.

•Numbers of myeloid cells were compared at different time points and after treatment.

Figure 1: Evolution of myeloid cells number in bone marrow, spleen and kidney after CTX treatment in mice inoculated with EL4.



CTX induces a depletion of myeloid cells in bone marrow, spleen and kidney of tumour bearing mice followed by an increase at D5 except for kidney macrophages that increase 24h after CTX treatment.

Aim 2: Role of CX3CR1 and CCR2 chemokine receptors on circulating myeloid cell numbers after CTX.

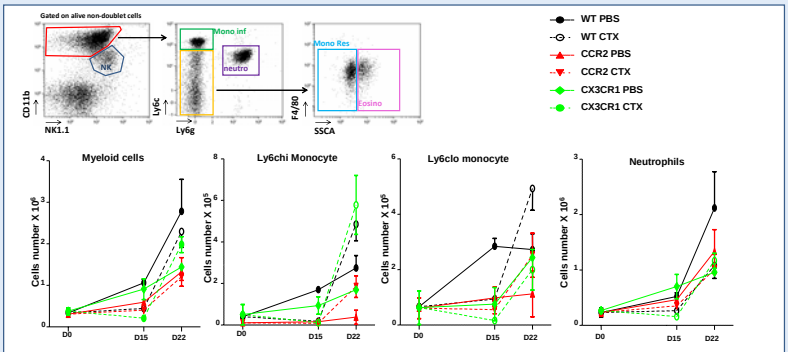
METHOD

•8-12 weeks old WT, CX3CR1-/- and CCR2-/- mice were injected iv with EL4 tumour cells and treated with CTX at D15.

•Blood was collected at D0, D16 and D22.

•Absolute numbers of myeloid cells were compared.

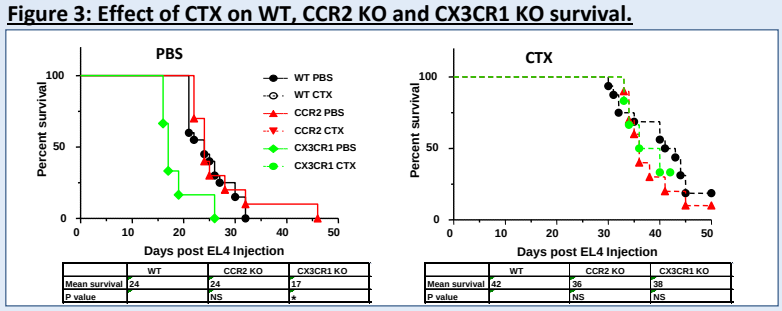
Figure 2:Effect of CTX treatment on circulating myeloid cell numbers in WT, CCR2 KO and CX3CR1 KO mice inoculated with EL4.



Tumour development is associated with a CCR2 dependant increase of Ly6chi monocytes and a CX3CR1 and CCR2 dependant increase of Ly6clow monocytes. Consistently, Ly6chi monocyte recovery after CTX is altered in the CCR2 KO mice while Ly6c low monocyte recovery is defective in both CX3CR1 KO and CCR2 KO mice.

Aim 3: Is the myeloid cells circulating number a prognosis marker of mouse survival ?

Figure 3: Effect of CTX on WT, CCR2 KO and CX3CR1 KO survival.

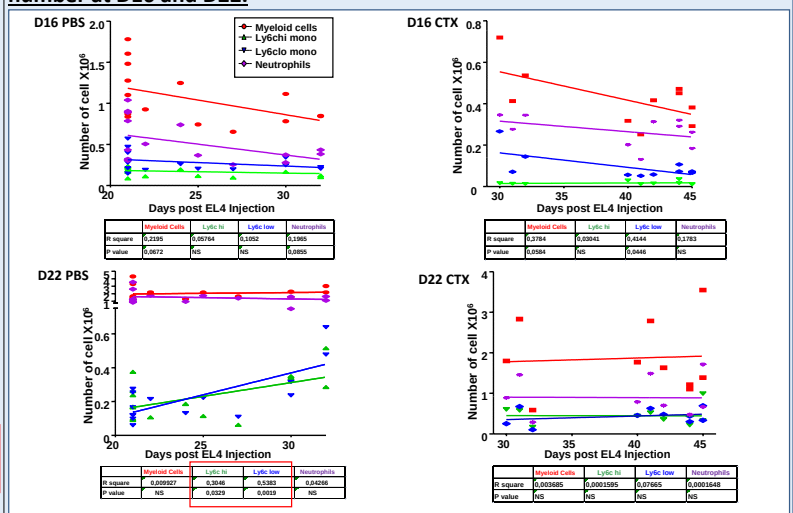


	WT	CCR2 KO	CX3CR1 KO
Mean survival	24	24	17
P value		NS	*

	WT	CCR2 KO	CX3CR1 KO
Mean survival	42	36	38
P value		NS	NS

Untreated CX3CR1 KO mice display decrease survival to systemic EL4 inoculation while CTX treated CX3CR1 mice have a similar survival to WT treated mice.

Figure 4: Correlation between WT mouse survival and circulating myeloid cells number at D16 and D22.



	Myeloid Cells	Ly6c hi	Ly6c low	Neutrophils
R square	0,2195	0,05764	0,1052	0,1965
P value	0,0672	NS	NS	0,0855

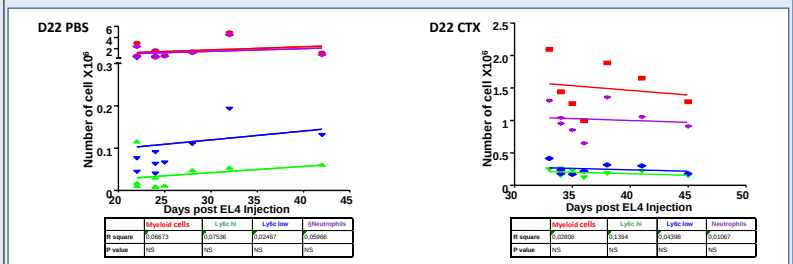
	Myeloid Cells	Ly6c hi	Ly6c low	Neutrophils
R square	0,3784	0,03041	0,4144	0,1783
P value	0,0584	NS	0,0446	NS

	Myeloid Cells	Ly6c hi	Ly6c low	Neutrophils
R square	0,009927	0,3046	0,5383	0,04266
P value	NS	0,0329	0,0019	NS

	Myeloid Cells	Ly6c hi	Ly6c low	Neutrophils
R square	0,003685	0,0001595	0,07665	0,0001648
P value	NS	NS	NS	NS

Ly6chi and Ly6clow monocyte numbers at D22 are positively correlated with mouse survival. This correlation is lost when monocyte number is artificially increased by CTX induced monocyte repopulation.

Figure 5: Predictive properties of circulating monocytes on mouse survival in CCR2 KO mice.



	Myeloid cells	Ly6c hi	Ly6c low	Neutrophils
R square	0,06673	0,07536	0,02467	0,05966
P value	NS	NS	NS	NS

	Myeloid cells	Ly6c hi	Ly6c low	Neutrophils
R square	0,02806	0,1394	0,04398	0,01067
P value	NS	NS	NS	NS

Predictive value of monocyte number is lost in untreated CR2 KO mice

CONCLUSIONS

•CTX differentially affects myeloid cells in the bone marrow, spleen, kidney and blood of EL4 bearing mice.

•Circulating myeloid cells recovery after CTX treatment is altered in CCR2 KO and CX3CR1 KO mice.

•CCR2 dependent predictive value of circulating monocyte number is lost after CTX treatment.

PERSPECTIVES

•Studying by intra vital microscopy the role of chemokine receptor expression in macrophage recruitment and behaviours in the tumour environment of PBS or CTX treated mice.

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