Autoimmunity and cancer immunosurveillance in the biliary tree

Juliette Paillet[1-3], Sarah Lévesque[1-3], Julie Le Naour[1-3], Céleste Plantureux[1-2], Gautier Stoll[1-2], Allan Sauvat[1-2], Pamela Caudana[4], Jimena Tosello Boari[4], Norma Bloy[1-3], Isabelle Martins[1-2], Paule Opolon[5], Agathe Delaune[6], Noémie Robil[6], Pierre de la Grange[6], Juliette Hamroune[7], Franck Letourneur[7], Patrick S.C. Leung[8], M. Eric Gershwin[8], Jie S. Zhu[8], Mark J. Kurth[8], Bouchra Lekbaby[9], Jérémy Augustin[9], Youra Kim[10], Shashi Gujar[10,11], Cédric Coulouarn[12], Laura Fouassier[9], Laurence Zitvogel[13], Eliane Piaggio[14], Chantal Housset[9,15], Patrick Soussan[9], M. Chiara Maiuri[1-2], Guido Kroemer[1-2,16-19], **Jonathan Pol**[1-2]

1 Centre de Recherche des Cordeliers, INSERM U1138, Université de Paris, Sorbonne Université, Paris, France
2 Metabolomics and Cell Biology Platforms, Gustave Roussy Cancer Campus, Villejuif, France
3 Université Paris-Saclay, Faculté de Médecine, Kremlin-Bicêtre, France
4 Institut Curie, PSL Research University, INSERM U932, Paris, France
5 Gustave Roussy Cancer Campus, Villejuif, France
6 GenoSplice Technology, Paris, France
7 INSERM U1016, Institut Cochin, Paris, France
8 University of California Davis, Davis, CA, USA
9 Centre de Recherche Saint-Antoine, INSERM U938, Sorbonne Université, Paris, France
10 Dalhousie University, Halifax, NS, Canada
11 Beatrice Hunter Cancer Research Institute, Halifax, NS, Canada
12 INSERM U1241, Université de Rennes, INRA, Institut NuMeCan, Rennes, France
13 INSERM U1015, Gustave Roussy Cancer Campus, Villejuif, France
14 Centre d'Investigation Clinique Biotherapie CICBT 1428, Institut Curie, Paris, France
15 AP-HP, Ref. Center for Inflammatory Biliary Diseases and Autoimmune Hepatitis, Saint-Antoine Hospital, Paris, France
16 Institut Universitaire de France, Paris, France
17 Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France
18 Suzhou Institute for Systems Medicine, Chinese Academy of Medical Sciences, Suzhou, China
19 Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden

Introduction

Primary biliary cholangitis (PBC)[1] and primary sclerosing cholangitis (PSC)[2] are two chronic inflammatory diseases of the biliary tract. Chronic inflammation is known to be one of the main risk factors for cancer onset. In this line, PSC is the first etiology of cholangiocarcinoma (CCA)[3] in Western countries. But surprisingly, patients with PBC close to never develop CCA. Since PBC harbors an autoimmune component, we hypothesised that PBC-associated autoimmunity could fuel cancer immunosurveillance and thus prevent CCA appearance.

1. Validation of the murine models of cholangitis

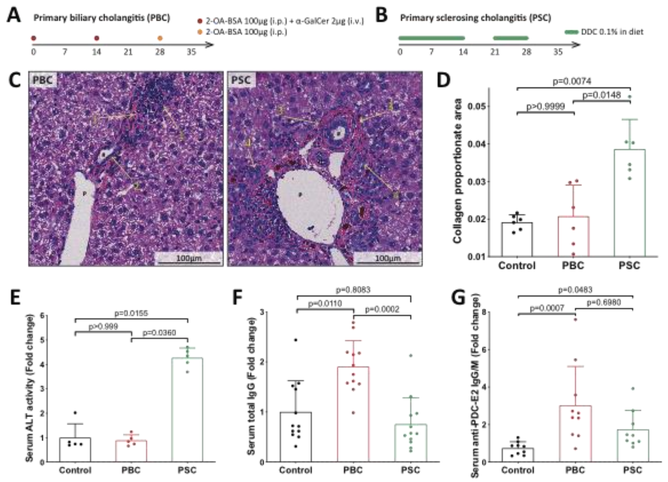

Figure 1. Experimental schemes of PBC **(A)** and PSC **(B)** induction in immunocompetent C57Bl/6 mice. **C.** Histological sections of liver at day 35 stained with hematoxylin, eosin and safran, p: portal vein, *: bile duct; 1: leukocytic infiltrate, 2: hyperplastic bile duct, 3: onion skin-like fibrosis, 4: ductular reaction. **D.** Collagen proportionate area measured on Syrius red-stained liver slices at day 35. **E.** Relative activity of alanine transaminase (ALT) measured in serum at day 35. Relative levels of total IgG **(F)** and IgG and IgM specific of PDC-E2 **(G)** measured in serum at day 35/36. **D-G.** Graphs show individual and mean (± SD) values. P-values were calculated by means of ANOVA test Tukey's pairwise multiple comparison.

References
1. Wakabayashi et al. Hepatology. 2008
2. Fickert et al. Am J Pathol. 2007
3. Yamada et al. Hepatology. 2015

2. PBC protects against CCA in a specific & T-cell dependent manner

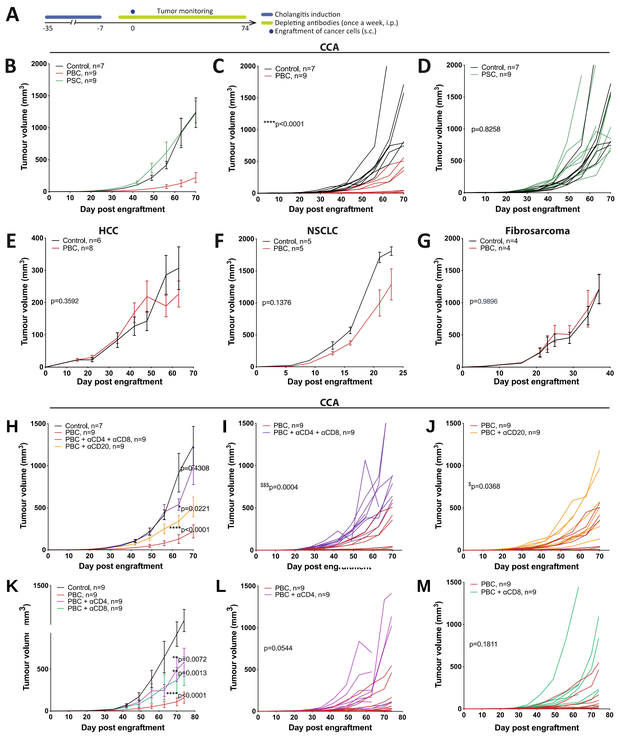

Figure 2. A. Experimental scheme. Growth of subcutaneous syngeneic CCA tumors in control, PSC or PBC mice **(B-D)** or PBC mice without or with injections of antibodies targeting either CD4 and/or CD8 or CD20, to deplete $CD4^+$ and/or $CD8^+$ T or B cells, respectively **(H-M)**. Growth of subcutaneous syngeneic tumors of hepatocellular carcinoma (HCC) **(E)**, non-small-cell lung cancer (NSCLC) **(F)**, and fibroscarcoma **(G)** in control and PBC mice. Graphs show mean (± SEM) **(B, E-H, K)** and individual **(C-D, I-J, L-M)** tumor growth curves. P-values were calculated by means of a linear mixed-effect model.

3. PBC protection against CCA relies on type-1/2 immune responses

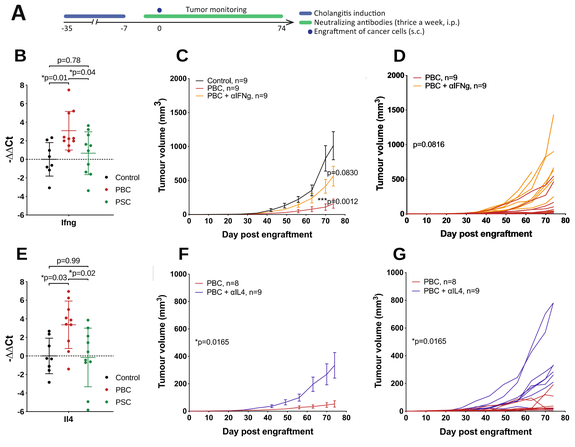

Figure 3. A. Experimental scheme. Relative expression of Ifng **(B)** and Il4 **(E)** genes measured by RT-qPCR within CCA tumors from control, PBC and PSC mice at day 56. Graphs show individual and mean (± SD) values. P-values were calculated by ANOVA with Tukey's pairwise multiple comparison. **C-D, F-G.** Growth of subcutaneous syngeneic CCA tumors in control and PBC mice, or PBC mice injected with antibodies targeting either IFNγ or IL4. Graphs show mean (± SEM) **(C, F)** and individual **(D, G)** tumor growth curves. P-values were calculated by means of a linear mixed-effect model.

4. Hepatic and CCA tumor tissues share similar enriched TCR clonotypes upon PBC

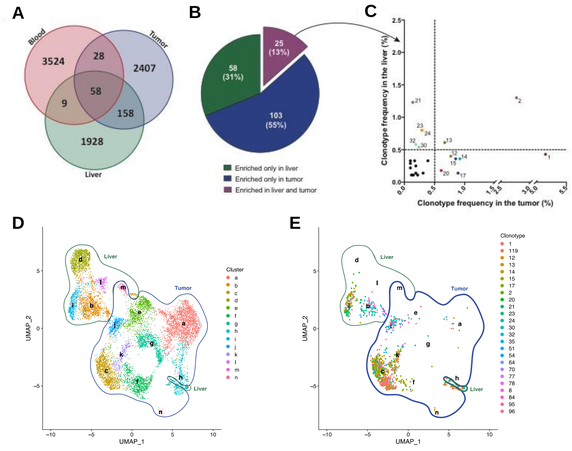

Figure 4. T cell clones infiltrating CCA, hepatic and blood tissues of a PBC mouse were characterized by single-cell TCR sequencing coupled with single-cell RNA-sequencing. **A.** Venn diagram showing the number of TCR clonotypes overlapping between tumor, liver and blood. **B.** Number of TCR clonotypes enriched (i.e. >0,1% of T cells) only in tumor (n=103), or liver (n=58), or both tissues (n=25). **C.** Proportion in the liver *versus* CCA tumor of each of the 25 TCR clonotypes enriched and shared between both tissues. **D.** Phenotypic clustering of total T lymphocytes sorted out from CCA and hepatic tissues of a PBC mouse. **E.** Phenotypic clustering of the 25 TCR clonotypes enriched in both CCA tumor and liver upon PBC.

Conclusion

Our data demonstrated that PBC protects against CCA outgrowth through an active mechanism mainly relying on both $CD4^+$ and $CD8^+$ T-cells, and in a lesser extent on B-cells. PBC-associated antitumor activity was specific to CCA. Type-1 and -2 immune responses, relying on the cytokines IFNγ and IL4 respectively, were mediating CCA immunosurveillance upon PBC. Finally, single-cell TCR/RNA-sequencing analyses revealed an enrichment of T cells with identical TCR in both liver and ectopic CCA tumor of a PBC mice. Altogether, our data provide mechanistic insights into an overlap between autoimmunity and cancer immunosurveillance in the biliary tree.