Characterization of anti-C5aR antibodies for specific targeting of myeloid cells and neutrophils in the TME

Poster # B184

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Background

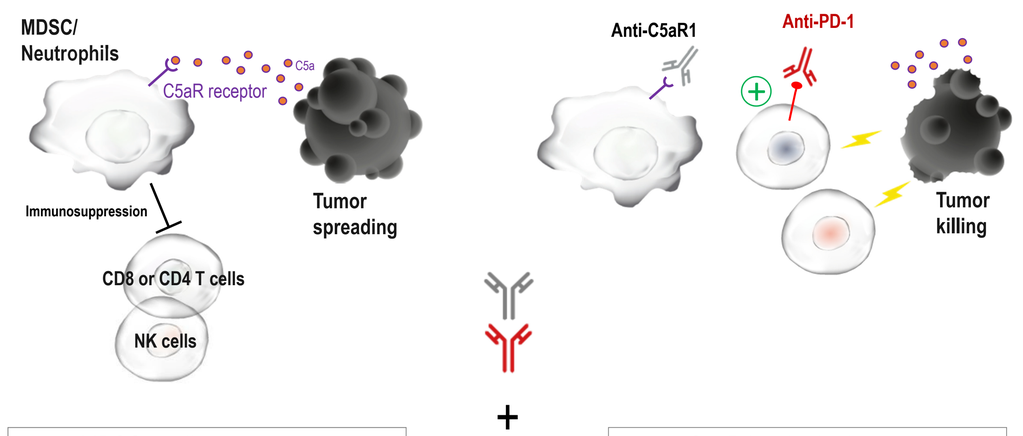
Accumulation within tumors of immunosuppressive myeloid cells and neutrophils is associated with poor prognosis in many cancers, as well as resistance to checkpoint blockade. A hallmark mechanism of synergy in immunotherapy is the elimination of these immunosuppressive cells to allow for the reactivation of effector cells. From a therapeutic perspective, we aimed to specifically target these suppressive mediators to impede their recruitment into the tumor microenviroment (TME) and promote a more potent antitumoral response.

The anaphylatoxin C5a, produced during the complement cascade, and its receptor (C5aR1) have been attractive therapeutic targets in autoimmune and inflammatory disorders. However, the active role of this pathway in tumor immune surveillance is only starting to come to light. In several murine cancer models, pharmacological blockade of C5a and in C5aR KO mice, tumors are reported to be infiltrated by markedly fewer myeloid derived suppressor cells (MDSCs), which produce less immunosuppressive cytokines, and are also functionally unable to suppress T and NK cells.

We confirm here the distinctive restricted expression profile of human C5aR1 on circulating immunosuppressive populations, and further describe the characterization of IPH5401, a novel fully human anti-C5aR1 antibody. IPH5401 specifically binds to hu-C5aR1 and prevents its binding to C5a. *In-vitro*, IPH5401 efficiently blocks C5a downstream events typical of neutrophil activation. We finally present in mice the anti-tumor efficacy of targeting the C5a/C5aR1 pathway in combination with PD-1 antibodies.

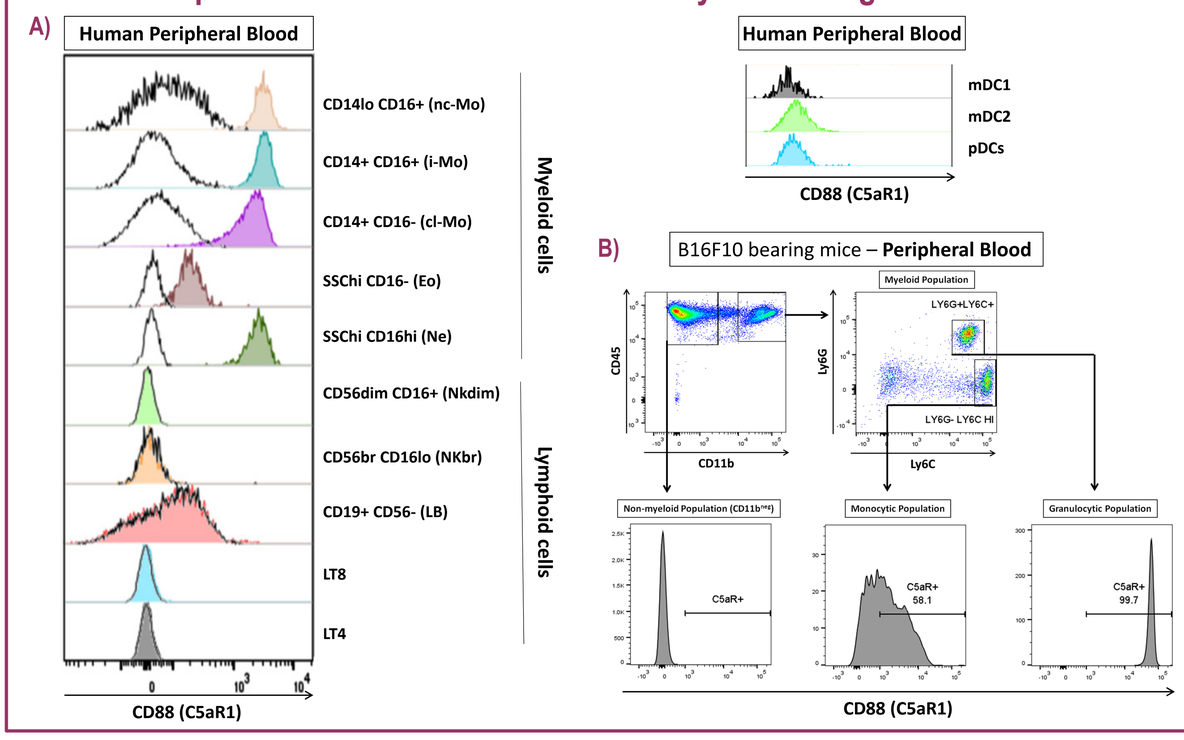
Mechanism of action

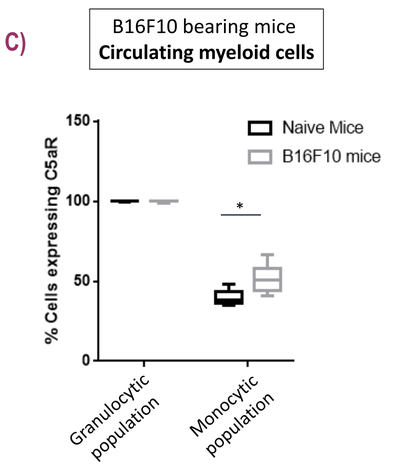
Combination therapy with anti-C5aR1 and anti-PD-1

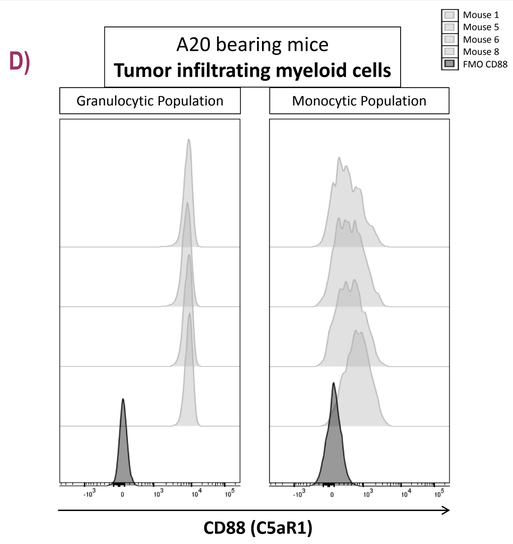


MDSC mediate pro-tumoral inflammation and immune suppression → C5aR blockade decreases MDSC infiltration and their immunosuppression of effector cells

Selective expression of C5aR1 on cells of the myeloid lineage

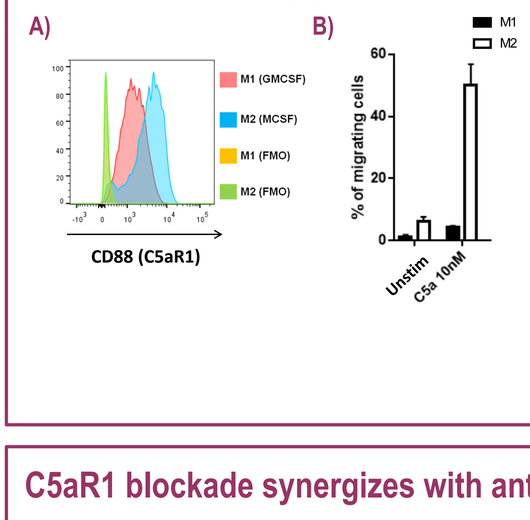






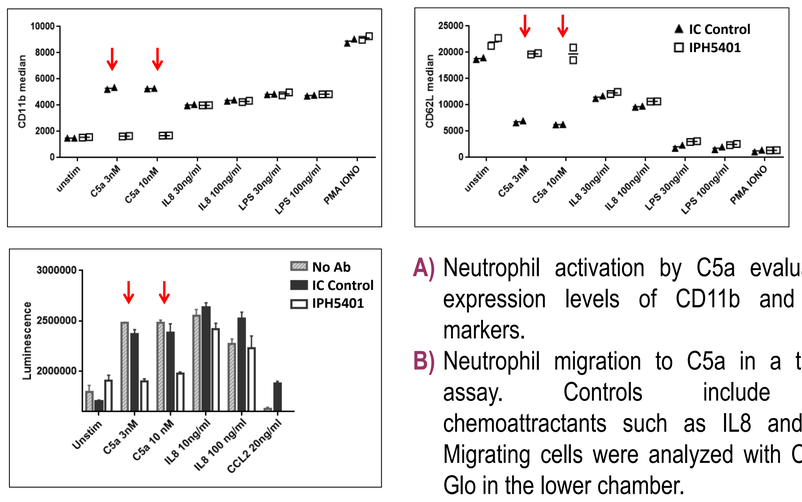
A) C5aR1 expression in the myeloid and the lymphoid populations of peripheral blood of healthy donors analyzed by FACS. Representative histograms from Donor DS271 are shown here. Black lines represent FMO staining.
B) C5aR1 expression in circulating myeloid and non-myeloid populations of mice bearing B16F10 tumors analysed by FACS. Peripheral blood was collected from the animals when the tumor volume was at 200mm^3.
C) FACS analysis of C5aR1 expression in peripheral blood of naïve and B16F10 bearing mice (n=5 and 7 respectively).
D) FACS analysis of C5aR1 expression in subcutaneously engrafted A20 tumors.

Immunosuppressive human M2 macrophages express high levels of C5aR1 and respond strongly to C5a-induced chemotaxis



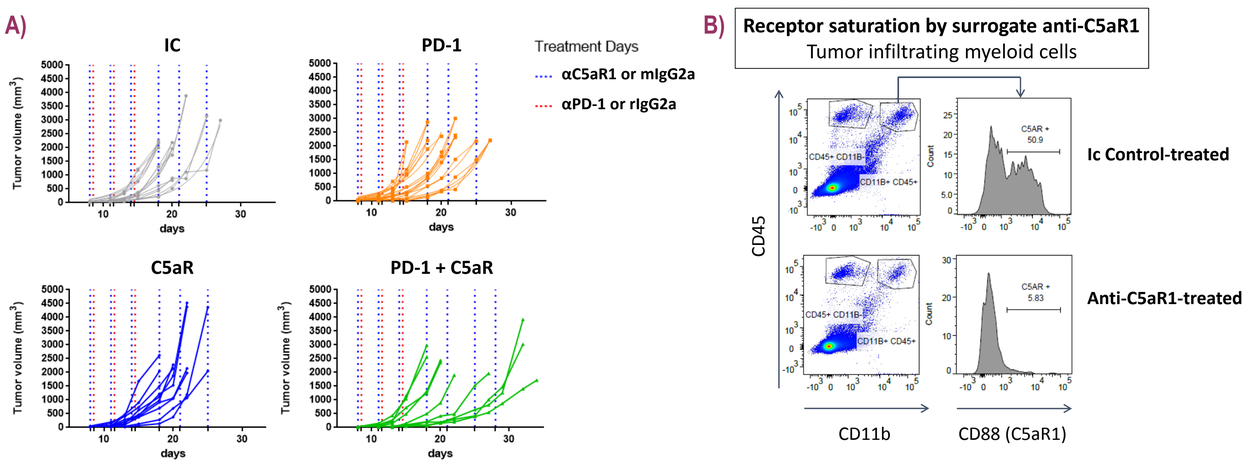
A) FACS analysis shows that M2-derived macrophages express higher levels of C5aR1 than M1-derived macrophages.
B) Increased migration of M2-derived macrophages to C5a compared to M1-derived macrophages in a transwell assay. Migrating cells were analysed with Cell Titer Glo in the lower chamber.
C) M2 cells respond strongly to C5a-induced chemotaxis compared to M1 cells in a transwell migration assay using 10nm of C5a as chemoattractant. M2 cells are labelled with CFSE and M1 cells are left unlabelled.

IPH5401 selectively inhibits C5a-induced activation and migration of human neutrophils



A) Neutrophil activation by C5a evaluated by expression levels of CD11b and CD62L markers.
B) Neutrophil migration to C5a in a transwell assay. Controls include other chemoattractants such as IL8 and CCL2. Migrating cells were analyzed with Cell Titer Glo in the lower chamber.

C5aR1 blockade synergizes with anti-PD-1 blockade to delay tumor progression



A) *Upper panels:* Mice (n=10 per group) were engrafted with B16F10 cells and treated with surrogate anti-C5aR and/or anti-PD-1, as indicated. Treatment days and frequencies are indicated.
Lower panel: Tumor growth inhibition percentage at the different tumor sizes. TGI is calculated based on the tumor volumes of the different treated groups reached once the control group reaches a predetermined volume (500, 1000, 1500, and 2000mm^3 shown here).
B) Surrogate Anti-C5aR1 saturates C5a Receptor on the tumor infiltrating myeloid cells. FACS analysis of the unbound C5a Receptor in the tumor infiltrating myeloid population. B16F10 tumors were collected and analyzed at day 16 (tumor size ~500mm^3).

Conclusions

- C5aR1 is selectively expressed on circulating human neutrophils and myeloid cell subsets.
- In tumor-bearing mice, C5aR1 is strongly expressed in both tumor infiltrating and circulating suppressive myeloid populations. C5aR1 expression is further upregulated compared to tumor-free animals.
- C5aR1 expression is higher in human M2-derived immunosuppressive cells compared to the non suppressive M1 subset.
- Accordingly to their high expression level of C5aR1, M2-derived macrophages present a stronger ability to migrate to C5a, suggesting a role for C5aR1 in the chemotaxis of myeloid cells to the tumor microenvironment.
- IPH5401, a fully humanized anti-C5aR1 antibody, effectively inhibits the C5a-mediated effects on neutrophil activation and migration.
- The combined administration of a surrogate anti-C5aR1 blocking antibody with anti-PD-1 synergistically reduced tumor growth in a poorly infiltrated tumor model *in-vivo*.

Overall, these data provide a strong incentive to clinically explore combination therapies with anti-PD-1 using a C5aR1 antibody. IPH5401 represents a unique opportunity to successfully reverse the tumor immunosuppressive microenvironment and overcome tumor resistance in cancer immunotherapy.

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