INTERACTION OF ANTI-PD-1/PD-1 IMMUNOCOMPLEXES WITH HUMAN FCγRS: BINDING PROPERTIES AND FUNCTIONAL IMPLICATION

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Background Rescue of exhausted T cell immunity through the inhibition of PD-1/PD-L1 interaction is a pillar of immunotherapeutic anti-PD-1 monoclonal antibodies (mAbs), as Nivolumab and Pembrolizumab. Despite the IgG4 subclass, anti-PD-1 mAbs can bind different FcγRs and trigger immunosuppressive activity in FcR-expressing myeloid cells. This effect is evident when anti-PD-1 mAbs engage soluble PD-1 (sPD-1) forming a stable sPD-1-antiPD-1mAb immune complex (PD-1 IC). In the present study we dissect the process by investigating which of the FcγRs displays the highest affinity for monomeric or complexed Nivolumab. In addition, methods for detecting and quantifying PD-1 ICs in plasma of patients treated with PD-1 blockers mAbs are under development for clinical application.

Methods By surface plasmon resonance (SRP) (Biacore™ T200, Cytiva), the interaction of FcγRI/CD64, FcγRIIa/CD32a, FcγRIIb/CD32b, FcγRIIIa/CD16a and FcγRIIIb/CD16b with anti-PD1 Nivolumab and the corresponding PD-1-IC has been evaluated, and their cellular localization has been assessed by confocal microscopy. Further, sPD-1 and PD-1 IC has been determined by customized ELISAs and western blot approaches in plasma of anti-PD-1 mAb-treated patients.

Results The binding of anti-PD1 and PD-1-IC occurred with all the FcγRs tested, albeit sensorgrams revealed diverse degrees of affinity. No major difference in the overall affinity of the monomeric anti-PD-1 versus PD-1 IC is observed for FcγRI/CD64, FcγRIIa/CD32a, FcγRIIb/CD32b. Instead, the dissociation phase was clearly slowest for PD-1 IC with respect to the monomeric mAb in FcγRIIIa/CD16a and FcγRIIIb/CD16b binding studies. Nevertheless, only PD-1 IC undergoes internalization when interacting with human FcγR+ myeloid cells in vitro, through pathways that does not appear to lead to lysosomal co-localization. Customized ELISA for PD-1 IC quantification has been developed to detect the complex after enrichment of plasma IgG4 by specific matrix. Data concerning the evaluation of PD-1 IC concentration in plasma of cancer patients treated with Nivolumab, will be presented.

Conclusions Despite the IgG4 subclass is expected to display the lowest binding affinity to Fcγs, we report here that anti-PD-1 therapeutic antibodies bind significantly to CD16, CD32 and CD64, particularly if stabilized in the IC form by engaging soluble PD-1. This evidence, if occurring in vivo, could introduce novel functional properties to these therapeutic agents, with potential detrimental effects on their clinical efficacy. Our findings imply that tools to antagonize PD-1-related exhaustion by Fc-null mAbs or non-mAb-based strategies could be preferred to engage full-fledged antitumor immune responses without unwanted effects related to FcR triggering.

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