Bispecific Antibody Constructs Mediate Immunotherapeutic Retargeting of Effector Cells Towards HBV Infected Target Cells

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Introduction

The immunotherapeutic retargeting of effector cells is a promising approach to circumvent the immunocompromised state found in malignancies and chronic viral infections. Effector cells are supplied with designed specificities and retargeted towards antigens reverting the immunocompromised situation.

To elaborate existing retargeting strategies, we constructed tetravalent bispecific antibody constructs harboring two different binding moieties of immunoglobulins. The first binding site is designed to target HBV-infected cells by binding the small HBV surface proteins on the plasma membrane of the infected cells. The second binding moiety engages immune effector cells with specificities for T cells (CD3-T cell receptor and CD28 for costimulatory signals).

HBV-infected hepatocytes carrying a cdDNA display viral surface proteins on their extracellular membrane. This occurs because the mature capsid buds into extracellular membranes of multivesicular bodies with inserted small, middle and large surface proteins for envelopment. Since this process does not consume all surface proteins, excess proteins reside in the MVB-membrane. In the course of virus transport processes necessary for export of viral particles these membraneous HBV surface proteins reach the extracellular membrane and are present on the surface of infected hepatocytes.

Experimental Background

In co-cultures of HuH7-transfected AHA7 target cells, expressing HBV-surface protein, the co-administration of FRM1 and bispecific antibody constructs resulted in target cell killing up to 80%. The antibody concentration dependence experiment in co-cultures of FRM1 and HuH7 target cells using anti-CD3 and anti-HuH7 constructs (C, and the anti-CD3(Inc) and anti-HuH7(Inc) constructs (B). In co-cultures of HuH7 producing AHA7 target cells and huH7-T cells, the bispecific antibody constructs showed the intracellular cytokine staining for IFN-γ and TNF-α to determine the functional properties of (CD3 CD8 T cells) or (CD8 T cells) over time. This showed a proliferation of effector cells by bispecific antibody directed against HBV surface proteins.

Bispecific antibody constructs mediate cytokytic elimination of HBV-positive and HBV-infected target cells with a polyfunctional T-cell activation.

In co-cultures of HuH7-infected HepAFG cells, also the single administration of one bispecific antibody lead to target cell elimination of up to 80%. The antibody concentration resulted in synergic enhancement in co-culture of HuH7-infected HepAFG cells with FRM1 and the respective bispecific antibody constructs directed towards T-cell antigens, activation of effector cells resulted in the specific cytokytic elimination of HBV-infected HepAFG cells (D).

Summary & Conclusion

The newly designed bispecific tetravalent antibody constructs are capable of binding to HBV surface proteins on the membrane of HBV-producing hepatocytes. The retargeting of T cells employing bispecific tetraevallent antibody constructs resulted in synergistic polyfunctional activation and cytoxicity upon co-culture with HBV-transfected AHA7 target cells. Furthermore, bispecific antibody constructs mediated specific elimination of HBV-infected HepAFG cells. The activation upon administration of CD3 and CD28 specific constructs was accompanied by cytokytic degradation and translocation of Lamp-1 to the cellular membrane of effector cells. In the future these new therapeutic tools have to be further evaluated, both in vitro and in vivo. For this purpose, the bispecific antibodies will be produced in high yields and purified and further tested for bioavailability, safety and efficacy.

Taken together, retargeting of immune effector cells towards HBV-infected cells using bispecific antibody constructs is a promising new therapeutic approach for the elimination of HBV infected hepatocytes in chronic hepatitis B.