

High-density lipoproteins delivering interleukin-15

Maria C. Ochoa, Ignacio Melero and Pedro Berraondo*

Division of Hepatology and Gene Therapy; Center for Applied Medical Research, and University Clinic; University of Navarra, Pamplona; Navarra, Spain

Keywords: apolipoprotein AI, high-density lipoprotein, interleukin 15, Sushi domain

Abbreviations: HDL, high-density lipoprotein; IL, interleukin; IL-2R β , IL-2 receptor β ; IL-15R α , IL-15 receptor α ; γ c, common γ chain

Circulating lipoproteins may offer interesting properties as therapeutic carriers for cytokines and hormones, in terms of both stability and bio-distribution. The fusion of apolipoprotein A-I with interleukin-15 (IL-15) targets the latter to high-density lipoproteins (HDLs). The bioactivity of this chimera can be further enhanced by creating triple fusions with IL-15 receptor α domain involved in IL-15 trans-presentation.

High density lipoproteins (HDLs) are natural nanoparticles specialized in the transport of lipid macromolecules through the organism.¹ The function of HDLs in mediating the reverse transport of cholesterol is well known, but other bioactive lipids are also carried in their hydrophobic core enveloped by lipoproteins. The most abundant proteins of HDLs are involved in lipid transport, but many other proteins use these nanoparticles as vehicles to circulate.² Recent experiments have shown that microRNAs may also circulate carried by HDLs.³

The properties of HDLs as natural vehicles have been exploited to deliver hydrophobic drugs to the liver or to tumors, as these tissues brightly express the main HDL receptor, scavenger receptor Type I (SB-RI).¹ Many clinically-relevant antitumor drugs have indeed been encapsulated in HDLs.⁴ More recently, small-interfering RNAs have also been encapsulated so to reach hepatocytes and tumor cells through an SR-BI-dependent mechanism.⁴

We have recently shown that the pharmacokinetic properties of HDLs can be conferred to therapeutic proteins by fusing them with apolipoprotein A-I, the most abundant HDL lipoprotein.² Cytokines stand out among the therapeutic proteins that can benefit from this approach.

Indeed, a strategy that increases the half-life of cytokines while concentrating their activity on specific target organs may conceivably improve their performance as therapeutic agents.

Interleukin-15 (IL-15) is one of the most promising cytokines for cancer immunotherapy.⁵ IL-15 is a four-helix bundle cytokine family member that induces the proliferation of natural killer (NK) cells and T lymphocytes. The intracellular signaling cascade that mediates the activity of IL-15 is triggered upon interaction with a heterotrimeric receptor composed of the IL-2 receptor β (IL-2R β), the common gamma chain (γ c) and the IL-15 receptor α (IL-15R α). The minimal region of IL-15R α required for the formation of a functional receptor is known as Sushi domain. Intriguingly, IL-15 and IL-15R α form complexes at the cell membrane that—upon cell-to-cell contact—induce the activation and proliferation of neighboring lymphocytes expressing IL-2R β and γ c upon, a phenomenon termed trans-presentation.

Following encouraging preclinical data in animal tumor models, Phase I clinical trials testing recombinant IL-15 in cancer patients are underway (NCT01369888, NCT01385423, NCT01021059, NCT01572493). However, two potential drawbacks of the bolus administration of recombinant IL-15

must be overcome. On the one hand, this approach might result in a highly oscillating pharmacokinetics, which in turn might sustain waves of toxicity and suboptimal efficacy in waves. On the other hand, the restricted expression of IL-15R α might prevent the activity of exogenous IL-15 even when administered at high doses.

To circumvent these limitations, we applied the apolipoprotein A-I fusion technology to IL-15. To select the best candidates and to analyze quickly the behavior of different chimeric molecules, we took advantage of hydrodynamic plasmic delivery. This technology relies on the administration of plasmids coding for the protein of interest under the control of a eukaryotic promoter in a high volume of saline over a short period of time. With this approach, the plasmid reaches 10–15% hepatocytes, in which the protein will be expressed for several days.⁶

We constructed an expression plasmid encoding IL-15 fused to apolipoprotein A-I.⁷ In order to enhance trans-presentation, a plasmid encoding the sushi domain IL-15R α was co-administered with the IL-15-coding plasmid. The amount of the IL-15 chimera in the serum was higher than that of non-stabilized IL-15 at all time points analyzed, and the fusion protein circulated as part of HDLs.⁷ The bioactivity of the IL-15 fusion protein was enhanced

*Correspondence to: Pedro Berraondo; Email: pberraondol@unav.es

Submitted: 12/22/12; Accepted: 12/23/12

Citation: Ochoa MC, Melero I, Berraondo P. High-density lipoproteins delivering interleukin-15. *Oncolmunology* 2013; 2:e23410; <http://dx.doi.org/10.4161/onci.23410>

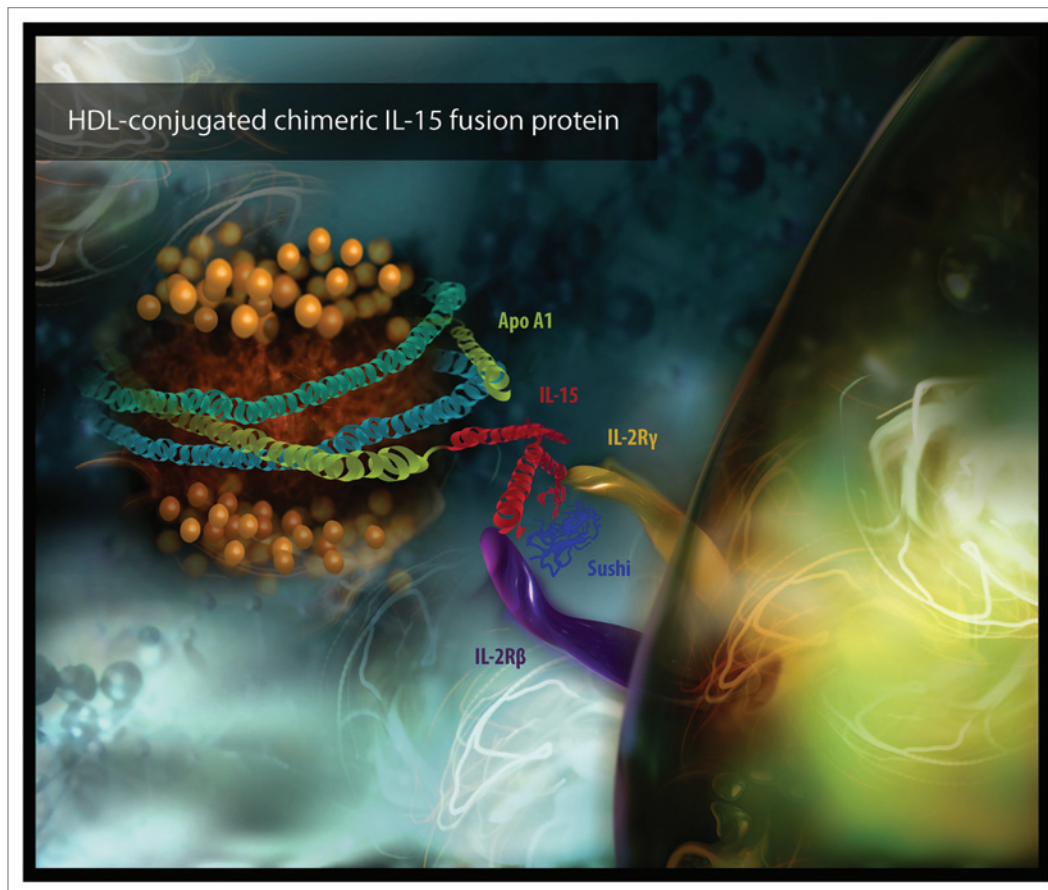


Figure 1. High-density lipoproteins as vehicles for interleukin-15. High-density lipoproteins (HDLs) can be used to trans-present a fusion protein involving interleukin-15 (IL-15) apolipoprotein A-I and the Sushi domain of the IL-15 receptor α (IL-15R α) to T lymphocytes or natural killer (NK) cells.

by the co-expression of the Sushi domain, increasing NK cell and memory CD8⁺ lymphocyte numbers in the peripheral blood, spleen and liver. Moreover, the administration of both plasmids partly rescued the NK-cell and the memory T-cell defects observed in the liver of *Il15ra*^{-/-} mice. However, the short half-life of the Sushi domain limited the therapeutic potential of this strategy, and we only observed a modest therapeutic activity against subcutaneously transplanted MC38 cell-derived colon carcinoma tumors.

To improve the stability of the Sushi domain, we constructed a plasmid encoding a triple fusion protein, combining apolipoprotein A-I, IL-15 and the Sushi domain (represented in Fig. 1 in its interaction with receptors on surface of a T lymphocyte or NK cells).⁸ This triple fusion protein was highly active and, following the hydrodynamic administration

of corresponding plasmid, mice died from acute lymphoid pneumonitis. The inflammatory infiltrates were mainly composed of T and NK cells. Experiments in genetically deficient mice allowed us to conclude that the toxicity was mediated by a mechanism involving a perforin- and granzyme A/B-dependent function mediated by activated NK cells. This acute toxicity illustrates the potent bioactivity of the triple fusion protein. Alongside, we were able to identify plasmid doses within the therapeutic range. These doses were well tolerated and promoted the proliferation of NK and memory CD8⁺ T cells in the spleen and liver. Moreover, the triple fusion protein exerted a potent antitumor activity against MC38 hepatic metastases as well as against lung metastases from ovalbumin-expressing B16 melanomas.⁸

In conclusion, the biodistribution of cholesterol-transporting HDLs offers an

interesting platform to deliver cytokines that control lymphocyte function, such as IL-15⁸ and interferon α .⁹ The triple fusion strategy advantageously modifies both pharmacokinetic and pharmacodynamic properties of this approach. Overall, the system exploits both the trans-presentation of IL-15 to antitumor lymphocytes (Fig. 1) and the particle-like nature of lipoproteins, offering a suitable delivery vehicle. Although we observed antitumor efficacy as monotherapy, we believe that the use of this approach in combination therapies will provide even more encouraging results. Experiments to explore the synergy of our chimeric molecules with other immunotherapeutic and non-immunotherapeutic anticancer agents are underway.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Trigatti B, Rigotti A, Krieger M. The role of the high-density lipoprotein receptor SR-BI in cholesterol metabolism. *Curr Opin Lipidol* 2000; 11:123-31; PMID:10787173; <http://dx.doi.org/10.1097/00041433-200004000-00004>
2. Vaisar T, Pennathur S, Green PS, Gharib SA, Hoofnagle AN, Cheung MC, et al. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. *J Clin Invest* 2007; 117:746-56; PMID:17332893; <http://dx.doi.org/10.1172/JCI26206>
3. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol* 2011; 13:423-33; PMID:21423178; <http://dx.doi.org/10.1038/ncb2210>
4. Damiano MG, Mutharasan RK, Tripathy S, McMahon KM, Thaxton CS. Templated high density lipoprotein nanoparticles as potential therapies and for molecular delivery. *Adv Drug Deliv Rev* 2012; In press; PMID:22921597; <http://dx.doi.org/10.1016/j.addr.2012.07.013>
5. Ochoa MC, Mazzolini G, Hervas-Stubbs S, de Sanmamed MF, Berraondo P, Melero I. Interleukin 15 in gene therapy of cancer. *Curr Gene Ther* 2012; In press. PMID:23157547
6. Arina A, Murillo O, Dubrot J, Azpilikueta A, Gabari I, Perez-Gracia JL, et al. Interleukin-15 liver gene transfer increases the number and function of IKDCs and NK cells. *Gene Ther* 2008; 15:473-83; PMID:18273053; <http://dx.doi.org/10.1038/gt.2008.4>
7. Ochoa MC, Fioravanti J, Duitman EH, Medina-Echeverez J, Palazon A, Arina A, et al. Liver gene transfer of interleukin-15 constructs that become part of circulating high density lipoproteins for immunotherapy. *PLoS One* 2012; 7:e52370; PMID:23285013
8. Ochoa MC, Fioravanti J, Rodriguez I, Hervas-Stubbs S, Azpilikueta A, Mazzolini G, et al. Antitumor immunotherapeutic and toxic properties of an HDL-conjugated chimeric IL-15 fusion protein. *Cancer Res* 2013; 73:139-49. PMID:23149919; <http://dx.doi.org/10.1158/0008-5472.CAN-12-2660>
9. Fioravanti J, González I, Medina-Echeverez J, Larrea E, Ardaiz N, González-Aseguinolaza G, et al. Anchoring interferon alpha to apolipoprotein A-I reduces hematological toxicity while enhancing immunostimulatory properties. *Hepatology* 2011; 53:1864-73; PMID:21425312; <http://dx.doi.org/10.1002/hep.24306>