Themes of thesis

Molecular adjuvants, or modulation of immune response by single chain fragments-mediated antigen targeting

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Introduction

Modern vaccine development focuses on the application of recombinant antigens in vaccines instead of using whole, fragmented or attenuated pathogens, because of their better safety. At the same time recombinant antigen-based vaccines proved to be less immunogenic compared to traditional vaccines and require adjuvant to induce and maintain protective antibody levels.

Molecular adjuvant, such as ligands of Toll-like receptors or agonistic antibodies, are able to induce local yet effective immune response without side effects due to their specific receptor or cell type targeting capability. One of the main group of molecular adjuvants is constituted by antibodies targeting co-stimulatory receptors or receptors involved in antigen uptake on antigen presenting cells (APC), which facilitates the development of effective immune response. Agonistic antibodies against complement receptor 1 and 2 (CR1/2), or against receptors recognizing Fc part of IgG with low affinity (FcγR II and III), and agonistic anti-CD40 antibodies belong to this group among others. Whole antibodies were usually used for targeting but they could induce inflammatory response or side effects since their Fc part could bind to FcγRs or activate complement system. Contrarily, the single chain fragments of antibodies (scFv) interact only the target

receptor due to loss of Fc-fragment, and possess low immunogenicity, thus only effect of targeted receptor prevails in immune response.

Nano-/ microparticles are proved to be effective adjuvant in enhancement of both humoral and cellular immunity. It has been described, that their immunogenic property relates with their size, because dendritic cells and macrophages prefer to uptake particles in viral or in bacterial size.

Aims

We have investigated the possible way of immunomodulation by scFvs. Our previous studies have shown that targeting model antigen to CR1/2 in mice with monomer scFv did not increase immune response against model antigen *in vivo*, despite of enhancement of antigen presentation *in vitro*. Our idea was that targeting antigen by oligomer scFv not only one –i.e. CR1/2- but more others main activating receptors of APC, such as FcγRII/III or/and CD40, in same time, can promote the development of effective immune response against the antigen. We planned to use streptavidin-biotin binding system for oligomerization of scFvs and thus for the base of our targeting

Further related publication

Scientific paper

- Adrienn Angyal**, Zsuzsanna Szekeres**, Péter Balogh, Zsuzsa Neer, Eszter Szarka, Viktor Virag, David Medgyesi, Jozsef Prechl, Gabriella Sarmay CD16/32 specific biotinylated 2.4G2 single chain Fv complexed with avidin-FITC enhances FITC-specific humoral immune response in vivo in a CD16-dependent manner International Immunology 2010 Feb;22(2):71-80.
- Papp K, **Szekeres Z**, Erdei A, Prechl J Two-dimensional immune profiles improve antigen microarray-based characterization of hum[illegible]mmunity. Proteomics, 2008 Jul ;8(14):2840-8.
- Papp K, **Szekeres Z**, Terenyi N, Isaak A, Erdei A, Prechl J. On-chip complement activation adds an extra dimension to antigen microarrays, Molecular & Cellular Proteomics 6: 133-140, 2007. Jan;6(1):133-40

Published abstract

- Erdei A, Molnár E, Isaak A, Papp K, **Szekeres Zs**, Papp K, Prechl J. Novel roles of CR1/2 on B lymphocytes. Molecular Immunology 44 (1-3): 166-166, Sp Issue 2007

- József Prechl, Eszter Molnár, **Zsuzsanna Szekeres**, Andrea Isaák, Krisztián Papp, Péter Balogh, Anna Erdei, Murine CR1/2 targeted antigenized single-chain antibody fragments induce transient low affinity antibodies and negatively influence an ongoing immune response. Advances in Experimental Medicine and Biology. 2007; 598:214-25.

Book lecture

- **Szekeres Zs,** Herbáth M, Prechl J., Immune response modulation by targeted complexes based on streptavidin, Biochemistry Research Updates, Nova Sciences Publichers, 2011 ISNB 978-1-61209-700-8

Published abstract:

- **Szekeres Zsuzsanna,** Herbath Melinda, Szittner Zoltán, Erdei Anna, Prechl József Modulation of immune response by combined targeting of complement receptors and low affinity Fcgamma receptors. European Journal of Immunology 2009, S532 (poster session), PD11/6,

complexes. Furthermore, we were also interested whether applying 510 nm microspheres in the targeting complexes, besides scFvs, can further increase the efficiency of scFv-mediated targeting and thus the immunogenicity of fused antigens.

Briefly our aims were

- to generate agonistic anti-CD40 FGK scFv, verify its specificity and analyze its binding capacity to APC.
- to create streptavidin-based targeting complexes with soluble streptavidin (SA) and scFvs, and then check their binding to the targeted cell population, such as B cells and macrophages.
- to create 510 nm microspheres-based targeting complexes (msSA-scFv) and characterize them in point of assembly, recognized by B cells and by macrophages, and *in vivo* localization.
- to influence the humoral immune response *in vivo* by targeting antigen to

  a, CR1/2 and/or FcγRII/III receptors

  b, CD40 and/or FcγRII/III receptors

  individually and in combination both with SA-scFv and msSA-scFv complexes.

Methods

- cloning of scFv, PCR, digestion, insertion into vector, transformation of bacterial cells, plasmid isolation, sequencing, agarose gel electrophoresis
- protein production by bacterial cells, protein purification, affinity chromatography
- biotinylation, labeling of antibody with fluorescent dye
- size exclusion chromatography,
- SDS-PAGE , dot plot,
- Flow cytometry
- Fluorescent microscopy
- cell culturing, animal maintenance and treatment, preparing cell suspension from spleen and lymph nodes
- ELISPOT, ELISA
- reverse protein microarray

Results

- We have cloned the variable region of light chain and heavy chain of FGK45.5 agonist anti-CD40 antibody, and generated FGK scFv using gene technologies. We have confirmed that

modulate the antigen specific humoral immune response. Thus, these targeting strategies could be suitable to increase immunogenicity of antigens or to fine regulation of humoral immune response as part of subunit vaccine.

Main publication related to this PhD study

Scientific paper

- **Zsuzsanna Szekeres**, Melinda Herbáth, Adrienn Angyal, Zoltán Szittner, Viktor Virág, Péter Balogh, Anna Erdei, József Prechl Modulation of immune response by combined targeting of complement receptors and low-affinity Fc-receptors Immunology Letters, 2010 May 4;130(1-2):66-73
- **Zsuzsanna Szekeres**, Melinda Herbáth, Zoltán Szittner, Krisztián Papp, Anna Erdei, József Prechl. Modulation of the humoral immune response by targeting CD40 and FcγRII/III; delivery of soluble but not particulate antigen to CD40 enhances antibody responses with a Th1 bias. Moleculare Immunology 2011 Sep 3. [Epub ahead of print]

Discussion

We have generated novel, modular targeting complexes with scFvs as targeting devices, which enables that only activation of targeted receptor involved in the modulation of the observed immune response. Furthermore, this modular structure of complexes gave possibility for combined receptor targeting, as well.

ScFvs on soluble complexes could efficiently deliver antigens to APCs and activated them. Antigen delivering to FcγRII/III or to CD40 in SA-scFv complexes was proved to be effective in enhancement of humoral immune response. In the case of targeting CD40 appearance of high amount of antigen specific IgG2a suggested Th1 bias in immune response. Combined scFv-mediated targeting of different receptors on APC did not shown their synergistic effect.

ScFvs were partially capable to direct 510 nm microspheres *in vitro*, but *in vivo* they failed to influence the induced immune response. Thus applying microspheres besides scFv in targeting complexes did not increase the efficacy of directing. Our data supported the adjuvant effect of the microspheres.

Our study has shown that targeting antigen to mFcγRII/III or to mCD40 by scFvs in soluble form is capable to enhance and

FGK scFv retained its original specificity, and was able to selectively recognize CD40 on APC.

- We have generated a modular targeting complexes via conjugation of monobiotinylated 7g6 scFv specific for mCR1/2, and/or 2.4g2 scFv specific for FcγRII/III, and/or FGK scFv against CD40 with soluble streptavidin (SA-scFv complexes) or with streptavidin-coated 510 nm microspheres (msSA-scFv complexes). In these complexes streptavidin and myc-tag and hexahistidin-tag fused to scFv served as model antigen. We have shown that SA-scFv complexes bound to B cells and to macrophages *in vitro* according to the specificity of the conjugated scFv.
- The msSA-scFv complexes have also bound to B cells and macrophages *in vitro*, but while their present on B cells reflected to binding ability of scFvs to these cell types, their binding on macrophages proved to be independent from that. We have also detected that all msSA-scFv complexes could reach draining lymph nodes after subcutaneous injection and localized mainly in their sinus network in the same way at each complexes.

- We have characterized the *in vivo* effects of targeting APC with SA-scFv or with msSA-scFv complexes on the development of antigen specific humoral immune response:
  - Targeting FcγRII/III both with SA-scFv and msSA-scFv complexes increased level of antigen specific IgG1 and IgG2a antibodies compared to only antigen containing control group.
  - Targeting CR1/2 receptors with soluble SA-7g6 constructs also enhanced the level of IgG1 antibodies against model antigens, compared to the control group, but the induced antibody response were lower then those induced by targeting of FcγRII/III. The msSA-7g6 complexes failed to elicit antibody response compared to control complexes. We have examined the complement activating property of 510 nm microspheres in sera to determine whether this observed weak immune response during antigen targeting to CR1/2 with msSA complexes might be originated by appearance of higher amount of C3 fragments that can mask the CR1/2. Data has shown that microspheres could slightly activate the complement system, but presumably the amount of released C3 fragments were not enough to weaken the efficacy of CR1/2 targeting.

  - Targeting antigens to CD40 with SA-FGK scFv complex has also enhanced the IgG1 antigen specific humoral immune response, and induced production of high amount of IgG2a antibodies, as well.
  - Combined scFv-mediated targeting of FcγRII/III and CR1/2, as well as FcγRII/III and CD40 receptors, did not caused further increasement in humoral immune response then targeting the FcγRII/III receptors alone.
  - The SA-scFv complexes elicited higher antibody response compared to control SA-myc-HH group, while the msSA-scFv complexes failed to do it compared to own control group. These data showed that scFvs can direct only the soluble streptavidin based complexes, in contrast with microspheres-based complexes.
  - Antigens conjugated to microspheres evoked elevated level of specific antibodies in sera, then antigens in soluble complexes. Furthermore, the microsphere-based targeting complexes induced an overall increase in antibody production.