Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Review

pH-dependent antigen-binding antibodies as a novel therapeutic modality☆

T. Igawa *, F. Mimoto, K. Hattori

Chugai Pharmaceutical Co. Ltd., Research Division, Japan

ARTICLE INFO

Article history:
Received 31 January 2014
Received in revised form 31 July 2014
Accepted 5 August 2014
Available online xxxx

Keywords:
Monoclonal antibody
Antibody engineering
pH dependent

ABSTRACT

Monoclonal antibodies have become a general modality in therapeutic development. However, even with infinite binding affinity to an antigen, a conventional antibody is limited in that it can bind to the antigen only once, and this results in antigen-mediated antibody clearance when the a membrane-bound antigen is targeted, or in antibody-mediated antigen accumulation when a soluble antigen is targeted. Recently, a pH-dependent antigen-binding antibody that binds to an antigen in plasma at neutral pH and dissociates from the antigen in endosome at acidic pH has been reported to overcome this limitation and to reduce antigen-mediated antibody clearance and antibody-mediated antigen accumulation. A pH-dependent binding antibody against a soluble antigen can be further improved by Fc engineering to enhance the Fc receptor binding. Various approaches, including histidine-based engineering, direct cloning from immunized animals, and synthetic and combinatorial libraries, have been successfully applied to generate pH-dependent binding antibodies against various antigens. This review discusses the features, approaches, advantages, and challenges of developing a pH-dependent binding antibody as a novel therapeutic modality. This article is part of a Special Issue entitled: Recent advances in molecular engineering of antibody.

1. Introduction

With more than 30 monoclonal antibodies already approved for therapeutic use [1] and more than 300 under clinical development [2], monoclonal antibodies are clearly expected to play an important role in future therapeutics. Therefore, antibody engineering technologies that improve the therapeutic potency of monoclonal antibodies have been extensively studied in this decade. Such technologies include improving the binding affinity to a target, the specificity, the pharmacokinetics, and the effector function mediated by the Fc region of an antibody. Binding affinity can be improved by several methods, such as *in vitro* affinity maturation by library display [3] and computer-based *in silico* design [4]. For pharmacokinetics, lowering the isoelectric point of the variable region of an antibody is reported to prolong the antibody half-life by reducing the non-specific clearance of the antibody [5]. Fc engineering to enhance the binding affinity to FcRn at acidic pH also improves pharmacokinetics by increasing the recycling efficiency from the endosomes after non-specific uptake into cells [6]. Modulation of the antibody effector function by optimizing the interaction between the Fc region and an Fc gamma receptor is reported to improve the therapeutic efficacy of a monoclonal antibody [7]. Another noteworthy technology that has recently been reported provides bispecific antibodies with a unique function that conventional monoclonal antibodies cannot achieve; they can simply neutralize two different disease-related cytokines [8]. In addition, bispecific antibodies can also redirect cytotoxic T cells to cancer cells by binding to CD3 and a tumor antigen [9], and they can mimic the function of coagulation factor VIII by binding to coagulation factor IXa and X [10].

Although these technologies have bestowed improved potency on therapeutic antibodies, it is now becoming more important to generate monoclonal antibodies that have further improved properties and are differentiated from conventional high-affinity monoclonal antibodies. In terms of modulating the interaction between an antigen and an antibody, achieving a high affinity binding to the antigen by affinity maturation has been the only approach for improvement. However, even with an infinite binding affinity to the antigen, a conventional antibody can bind to the antigen only once during its lifetime in plasma, and it is fundamentally limited by its consequent inability to neutralize further antigens, if the *in vivo* molar amount of antigen is larger than the amount of antibody injected.

If an antibody is targeting a membrane-bound antigen with a high rate of synthesis, such as IL-6 receptor (IL-6R), EGFR, CD4, or CD40 [11–14], the antibody is rapidly eliminated from plasma through antigen-mediated clearance. As a result, targeting these types of membrane antigen cannot be improved by affinity maturation and requires a high dose. Similarly, a high antibody dose is also required to neutralize soluble antigen at a high plasma concentration. The plasma

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* Corresponding author. Tel.: +81 550 87 3029; fax: +81 550 87 5326.
E-mail address: igawatmy@chugai-pharm.co.jp (T. Igawa).

http://dx.doi.org/10.1016/j.bbapap.2014.08.003
1570-9639/

concentration of soluble antigen after antibody administration is determined by two factors: the baseline concentration and the antibody-mediated antigen accumulation. Some soluble antigens, such as IgE and C5, have a plasma baseline concentration that is already very high [15,16]; other soluble antigens without a high baseline concentration in plasma accumulate more than 1000-fold because the recycling function of an antibody inhibits degradation of the antigen and results in a very high concentration [17]. This accumulation after antibody administration occurs because an antigen in complex with an antibody has a longer half-life than the antigen alone [18,19]. The resulting high plasma antigen concentration requires a high antibody dose to neutralize the antigen. For both membrane-bound and soluble antigens, the limitation of conventional antibodies in only binding to the antigen once, even when the binding affinity is infinite, requires a high antibody dose.

Recently, we and others have reported that a pH-dependent antigen-binding property could overcome this limitation of conventional antibodies [20–22]. An antibody with a pH-dependent antigen-binding property dissociates the bound antigen in acidic endosomes after internalization into cells. Consequently, the dissociated antigen is trafficked to the lysosome and degraded, whereas the dissociated antibody, free of antigen, is recycled back to plasma by FcRn. The recycled free antibody can bind to another target antigen. By repeating this cycle, a pH-dependent antigen-binding antibody can bind to the target molecule more than once. Moreover, we have shown that the therapeutic potency of a pH-dependent antigen-binding antibody can be further enhanced by increasing its binding affinity to FcRn at neutral pH [23]. These studies demonstrate that pH-dependent antigen-binding antibodies can overcome the limitation of conventional antibodies and allow novel antibody therapeutics with differentiating properties to be generated. In this review, the features, advantages, and challenges of pH-dependent antigen-binding antibodies, and how they can be generated and optimized are discussed.

2. Effect of a pH-dependent antigen-binding antibody

2.1. Against a membrane-bound antigen

Fig. 1A describes the fate of a conventional antibody bound to a membrane-bound antigen. A conventional antibody bound to the cell surface antigen is internalized into cells, after which the antibody–antigen complex passes through the sorting endosome and is transferred to a lysosome, where eventually both the antibody and antigen are degraded by proteolysis. This means that, even with infinite binding affinity to the antigen, a conventional antibody is limited to binding to an antigen only once. Conventional antibodies against membrane-bound antigens, such as IL-6R, EGFR, CD4, and CD40, exhibit non-linear clearance *in vivo* through antigen-mediated antibody clearance [11–14]. When a membrane-bound antigen is highly expressed in the body, the antibody is rapidly cleared from plasma, thereby requiring a high dose to neutralize this type of antigen over a long period.

Fig. 1B describes the fate of a pH-dependent antigen-binding antibody, which we also refer to as a recycling antibody, bound to a membrane-bound antigen. The recycling antibody bound to the cell surface antigen is internalized into the cells in the same way as a conventional antibody. However, in the sorting endosome, where the pH of the vesicle is acidic, the pH-dependent binding antibody is dissociated from the antigen. The antigen is transferred to the lysosome and degraded by proteolysis, while the dissociated antibody binds to FcRn in the endosome and is recycled back to the cell surface. Since FcRn does not bind to the antibody at neutral pH, the antibody dissociates from FcRn at the cell surface and returns back to plasma. A recycling antibody exhibits less non-linear antigen-mediated antibody clearance *in vivo* than conventional antibody and shows prolonged pharmacokinetics by avoiding antigen-mediated clearance. By repeating this cycle of binding at the cell surface and dissociating within the endosomes, a recycling

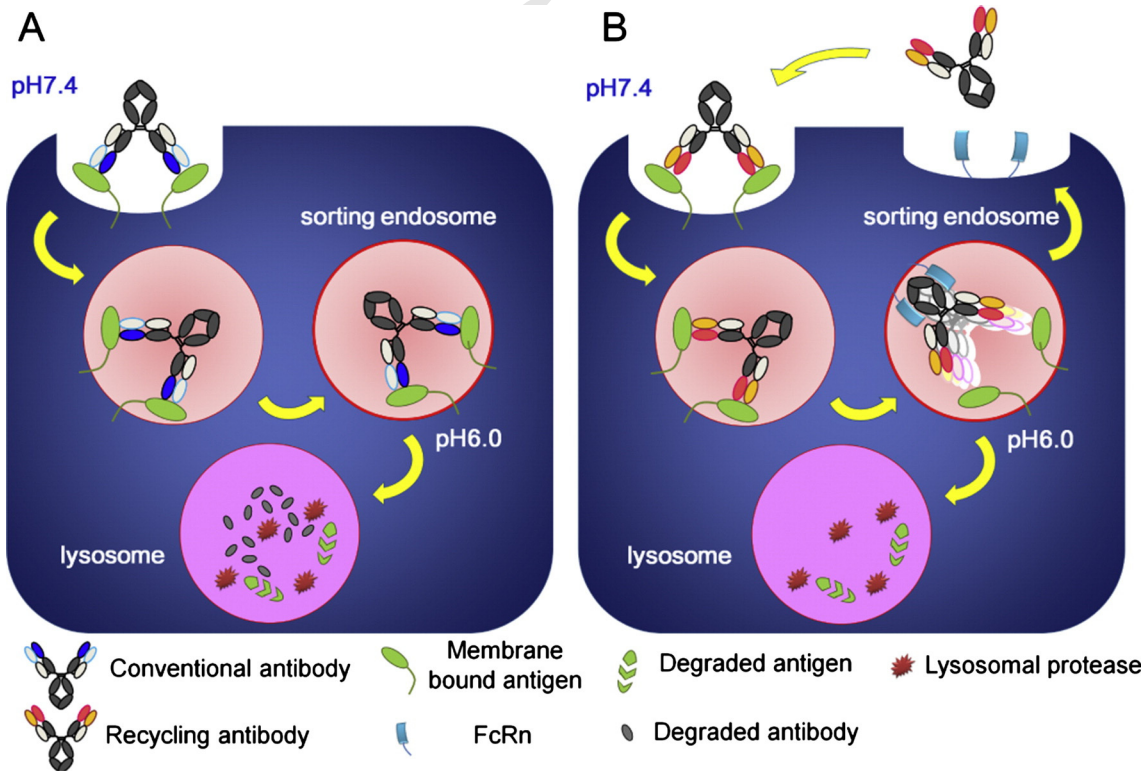

Fig. 1. Models showing the fate of (A) a conventional antibody and (B) a recycling antibody bound to a membrane-bound antigen. (A) A conventional antibody bound to a membrane-bound antigen is internalized into the cell, after which the antibody–antigen complex is trafficked to the sorting endosome and finally to lysosome, where eventually both the antibody and antigen are degraded by lysosomal protease. (B) A recycling antibody bound to a membrane-bound antigen is internalized into the cell in the same way as a conventional antibody. However, in the sorting endosome, where the pH of the vesicle is acidic, the recycling antibody is dissociated from the antigen by means of its pH-dependent antigen-binding property. The antigen is transferred to the lysosome and degraded by proteolysis, while the dissociated antibody is recycled back to the cell surface and plasma by utilizing FcRn.

Please cite this article as: T. Igawa, et al., pH-dependent antigen-binding antibodies as a novel therapeutic modality, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbapap.2014.08.003