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MONOCLONAL ANTIBODIES AS CARRIERS FOR DELIVERING CYTOTOXIC AGENTS

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Summary. - *Because of their high level of specificity monoclonal antibodies can be used as carriers for delivering cytotoxic agents. However they need to have some other features to be usefully used for this purpose. First of all monoclonal antibodies need to be specific for those membrane antigens which are strongly expressed on target cells but absent from normal cells. Secondly it is important to obtain monoclonal antibodies with as a high affinity as possible, and the biodistribution of the molecule recognized by the monoclonal antibody must be considered with the best attention. Chemical treatment of monoclonal antibodies needs to be performed in such a way that neither the antibody nor the toxic agent will result denaturated. Finally, as an alternative to chemically linked immunoconjugates, bispecific antibodies have successfully been used for delivering cytotoxic agents.*

KEY WORDS: monoclonal antibodies, cytotoxic agents, bispecific antibodies.

Riassunto (Anticorpi monoclonali come trasportatori di agenti citotossici). - *Grazie alla loro grande specificità gli anticorpi monoclonali possono essere usati come carrier di farmaci citotossici. Per questo debbono soddisfare alcune caratteristiche: innanzitutto debbono essere estremamente specifici per la cellula bersaglio. Per questo debbono essere usati anticorpi diretti contro antigeni di membrana non espressi in cellule normali ed espressi in alta densità su tutte le cellule bersaglio. L'affinità dell'anticorpo per l'antigene deve essere la più alta possibile e la biodistribuzione dell'anticorpo deve essere valutata attentamente. Inoltre la coniugazione chimica deve essere blanda in modo da non denaturare né l'antigene né l'anticorpo. Sono stati infine prodotti anticorpi monoclonali bispecifici in grado di veicolare efficacemente l'agente citotossico sulla cellula bersaglio.*

PAROLE CHIAVE: anticorpi monoclonali, agenti citotossici, anticorpi bispecifici.

Introduction

The major problem related to the use of drugs in anticancer chemotherapy is the lack of specificity for tumor cells, which determines a low ratio between therapeutic efficacy and systemic toxicity. Therefore, any approach capable of directing drugs more specifically against the tumor offers new opportunities to improve therapeutic applications. Ever since their development 13 years ago, monoclonal antibodies (MoAbs) have been considered potential carriers by many investigators, not only for drugs such as adriamycin or methotrexate, but also for radioisotopes such as iodine or yttrium, or for highly toxic proteins such as ricin [1].

Necessary characteristics for a good toxic-agent carrier

Reactivity directed against tumor cells is the first requirement, but many other conditions related to the target antigen, or inherent to the intrinsic peculiarities of the antibody are necessary for the MoAb in order to be a good carrier of toxic agents [2].

Target antigen

First of all the target antigen must be as specific as possible for the tumor cells. However, even MoAbs directed against molecules which are also present on normal cells have been used successfully *in vivo* to treat tumors. Quantitative rather than qualitative differences in antigen espression between normal and tumor cells are being exploited. Besides the tumor specificity the target antigen must be a membrane molecule and the antigenic determinant recognized by the MoAb must be present in the extra cellular domain of the molecule in order to be reached by

the plasma proteins. Even the number of antigenic determinants per tumor cell is relevant in order to concentrate both the MoAb and the toxic agent on the membrane. The behaviour of the antigen-antibody complex after antibody binding has been shown to be also important for cytotoxicity induced by some of the agents that can be transported by the antibody. For ribosome inactivating proteins the internalization process of the antigen antibody complex is a fondamental step to obtain the killing of the target cells, whereas for chemotherapeutic drugs no data concerning the involvement of the immunoconjugate processing has been reported. On the contrary, for radioisotopes such as iodine 131 as the toxic agent, not only is the internalization of the radiolabelled antibody inside the cell unnecessary, but it has been shown to be harmful (the free iodine is quickly eliminated by the cell). The requirement for an antigen expression homogeneity on the tumor cells also depends on the active mechanism of the cytotoxic agent. In fact, for cytotoxic agents which require MoAb internalization within the cell in order to be active, only cells expressing the antigen can be killed; whereas on the contrary, if no MoAb processing is required, the cytotoxic agent delivered on the membrane of one antigen positive cell can also kill the adjacent cells, which can be negative for the relevant antigen.

MoAb characteristics

As far as the intrinsic characteristics of the MoAb are concerned, the IgG isotype seems to be the most appropriate, even though experiments with IgM MoAbs have not been carried out extensively enough to definitely rule out their suitability as toxic agent carriers.

As regards the affinity, theoretically the highest possible level of affinity is required. However, this depends on the tumor specificity of the target antigen and in particular on the quantitative differences in expression between normal and tumor tissues. A MoAb with low affinity was used for *in vivo* therapy without side effects and in some cases resulted therapeutically useful. Moreover, when a high-affinity MoAb directed against the same antigen was used in the attempt to increase the therapeutical potential of the treatment, strong side effects due to the damage to normal epithelial cells, were observed.

Biodistribution of the MoAb

Many different *in vivo* therapeutic approaches have been reported in which MoAbs were used to transport different cytotoxic agents, mainly radioisotopes and toxins, and since the first clinical trials two major problems have emerged. The first one regards the *in vivo* biodistribution of the MoAb which, despite its fine specificity, always shows a first non-specific retention in some normal organs (in the liver and in some cases also in the spleen and in the bone marrow), followed by a more tumor specific phase [3]. The second problem concerns the chemical linkage between the antibody and the toxic agent. In many cases the methodology used for cross-linking may be denaturating for the molecules [4] and often the *in vivo* stability of the bridge is questionable.

Bifunctional MoAbs

As an alternative to chemically linked immunoconjugates, another potentially effective approach is the use of bispecific or bifunctional antibodies generated by linking together two MoAbs with the desired specificity [5]. The specific delivery of the functional agents to the target cells can be achieved via the innate ability of this hybrid antibody to bind simultaneously to both the target cell antigen and the cytotoxic agent. These hybrid MoAbs can be obtained chemically or by the fusion of two pre-existing hybridoma cells which produce the two MoAbs. This results in a hybrid hybridoma, or quadroma cell, in which the hybridoma immunoglobulin chains recombine to form a bifunctional MoAb. For this approach MoAbs directed against cytotoxic agents are required, which in some cases can be problematic. In fact, for some cytotoxic substances such as adriamycin, the production of MoAbs has only been possible after adaptation of the immunization schedule [6]. In fact, long-term immunization, commonly used to produce MoAbs, was found to be unsuitable for this goal since immunodepression rather than immune response was induced. Instead of using MoAbs directed against the toxic agent itself an "anti-hapten" MoAb may be used. The hapten can be conjugated to many different functional agents and therefore, the hybrid MoAb with both anti-tumor and anti-hapten specificity can be used as a unique reagent for different diagnostic and therapeutic approaches.

Many researchers are currently testing the utility of bifunctional antibodies in *in vivo* preclinical models. Recently it has been demonstrated in a murine model that with a bifunctional MoAb a specific localization of vinca alkaloids can be achieved in the tumor, thus resulting in an increase of the specific drug activity [7].

Conclusions

MoAbs has been demonstrated to be suitable carriers for cytotoxic agents as long as they are adequately selected for this application and novel approaches such as the use of hybrid MoAbs instead of immunoconjugates are opening up some very promising areas of investigation.

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