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Engineering T Lymphocyte Antigen Specificity

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Abstract Targeting of immune cells by bispecific antibodies has proven to be a powerful tool for the investigation of cellular cytotoxicity, lymphocyte activation and induction of cytokine production, as well as to represent an innovative form of immunotherapy for the treatment of cancer. The hallmark of this approach is the use of the specificity of monoclonal antibodies to join target and immune cells by virtue of the dual specificity of bispecific antibodies for the two entities. More precisely, the bispecific antibody has two different binding sites, which are capable of recognizing tumor associated antigens on the one hand and lymphocyte activation sites on the other. This process of crosslinking results in the activation of the lymphocyte and triggering of its lytic machinery, as well as lymphokine production. A major advantage of this therapeutic modality is, that use is made of the normal cellular immune defence system and therefore is only associated with minor toxicity. The distinct lymphocyte populations, which can be used for adoptive immunotherapy and the various bispecific antibody preparations, as well as the chimeric immunoglobulin/T cell receptor construction, are the major topics of this review.

Key words: T lymphocytes, bispecific antibody, cancer therapy

TUMOR ANTIGENICITY ELICITING IMMUNE REACTIVITY

Experimentally induced tumor cells can express "new antigens" (Ag) on their surface. Such Ag are unique or selective, may be immunogenic, and thus elicit a tumor specific cytotoxic T lymphocyte (CTL) response. The study of tumor-specific immune reactions was stimulated by the postulation of immune surveillance, as formulated by Ehrlich in 1909 [1] and adapted by Burnet in 1970 [2]. The keynote of the hypothesis is that antigen-specific T lymphocytes are the critical surveillance cells that arrest or eliminate cancer cells from the host. In general, specific T cells recognize their antigen in the context of the major histocompatibility complex (MHC) on the surface of the Ag presenting cell. In experimentally induced tumor models such MHC-restricted tumor-specific T-cells have been identified. In humans, "tumor-specific" CTL which are not MHC-restricted have been demonstrated [3,4]. Importantly, there appears to be a correlation between autologous tumor cell killing activity and prognosis (e.g., in lung cancer) [5]. The discovery of host antitumor immune reactivity has stimulated further extensive investigations in preclinical and clinical settings.

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WAVES OF IMMUNOTHERAPY

The first wave of immunotherapy started to roll at the end of the 19th century. It was William Coley who used anticancer "vaccines" consisting of bacteria. However, this immune approach was eclipsed by the advent of radiotherapy. A second wave then occurred in the late 1960s, and early 1970s, when certain bacteria, notably *Bacillus Calmette-Guérin* (BCG) and *Corynebacterium parvum* (*C. parvum*) were used for the treatment of cancer. These bacteria acted as adjuvants by nonspecifically stimulating cellular and humoral immune reactivity. Meanwhile, the demand for highly purified biological response modifiers (BRM), with better defined modes of action, became stronger.

The extension of our knowledge of the immune system to the molecular level has led to the identification, cloning, and large-scale production of a multitude of cytokines, while the advent of large-scale immune cell culture techniques allows the ex vivo activation and expansion of patients' lymphocytes for their clinical application. In addition, the availability of monoclonal antibodies (mAb) that bind selectively to tumor cells has allowed novel therapeutical strategies.

It is the synergism of these disciplines that gives way to the engineering of lymphocyte specificity. The present state-of-the-art technol-

ogies are continuously being translated into clinical treatment protocols for the immunotherapy of cancer: the third wave of biotherapy.

LYMPHOCYTE SUBSETS WITH LYTIC CAPACITY

Natural Killer Lymphocytes

MHC-unrestricted, "spontaneous" cytolysis of tumor cells by lymphocytes is described as "natural killer" (NK) activity. NK lymphocytes do not express a T cell receptor (TCR)/CD3 complex, implying that non-TCR structures must be involved in MHC-unrestricted cytolysis (see below). Their lytic activity is, by definition, MHC-unrestricted. This feature, together with the fact that NK cells exert lysis without prior activation requirements, represent the fundamental differences to the antigen-specific cytolysis displayed by TCR^+ lymphocytes [6,7,8,9].

T Cell Receptor Positive Lymphocytes

The TCR^+ lymphocyte population comprises two distinct lymphocyte subsets which are both capable of exerting cell-mediated cytotoxicity: T lymphocytes that express an antigen receptor, that is a disulphide-linked heterodimer composed of an α and a β protein [6], and T lymphocytes with an antigen-receptor composed of a γ and a δ protein. The γ and δ chains can be either disulphide or nondisulphide linked [10,11].

The specific antigens for MHC-restricted CTL are presented by class I or II molecules. TCR$\alpha\beta$ lymphocytes represent the vast majority ($> 90\%$) of peripheral-blood antigen-specific T lymphocytes and are either $CD4^+$ or 8^+. The $CD4^+$ lymphocyte subset acts as helper or cytotoxic cell and is restricted by MHC class II Ag presenting molecules on the target cells. The $CD8^+$ lymphocyte subset represents cytotoxic T lymphocytes that are restricted by MHC class I Ag presenting molecules [6]. After recognition of Ag, helper/inducer lymphocytes become activated and secrete a variety of growth and differentiation factors, such as the interleukins, to facilitate the antibody and/or CTL responses. $CD8^+$ CTL are involved in the elimination of host cells, expressing non-self antigens. Following, appropriate activation, these $CD4^+$ and $CD8^+$ specific CTL can also exert MHC-unrestricted lysis, probably involving non-TCR structures (see below).

The minor TCR$\gamma\delta$ lymphocyte fraction, (3–4% of the TCR^+ lymphocytes) virtually all lack the expression of CD4 or CD8 antigens [10–13].

Freshly isolated TCR$\gamma\delta$ lymphocytes do not lyse NK-susceptible target cells. Upon in vitro activation, TCR$\gamma\delta$ lymphocytes can exert MHC-unrestricted cytolysis towards a variety of tumor cells of distinct histologic origin, which does not involve the TCR. Antigen-specific TCR$\gamma\delta$ lymphocytes have also been identified. Some of the identified Ag are CD1c, TCT-1, HLA-A2, A23, DR7 and Dw6 [14]. TCR$\gamma\delta$ lymphocytes can be divided on the basis of their functional rearrangements into two distinct subsets. The Vγ9-Vδ2 encoded TCR$\gamma\delta$ heterodimer is the predominant receptor type in peripheral blood, whereas the Vδ1 encoded TCR$\gamma\delta$ heterodimer represents a minority [13]. Both subsets can produce a variety of lymphokines.

RECEPTORS INVOLVED IN CYTOTOXICITY

The T Cell Receptor

The T-lymphocyte structure capable of specific antigen recognition is the membrane-expressed polymorphic TCR [15,16]. The TCR is noncovalently associated with the nonpolymorphic CD3 complex that comprises five different proteins. The TCR genes contain segments homologous to the variable (V), diversity (D), joining (J), and constant (C) gene segments of the immunoglobulin (Ig) gene. Thus, antigen-specific cellular cytotoxicity is exerted by lymphocytes that express a surface TCR capable of discriminating between "foreign" cells and normal autologous cells. The binding of these Ag specific TCR^+ lymphocytes to their target cell generates an activation signal.

Non-TCR Lymphocyte Surface Receptors Involved in MHC-Unrestricted Cytolysis

Nonpolymorphic glycoproteins, such as CD2, CD3, CD16, CD28, are also involved in lymphocyte activation and triggering for lysis. Some of these structures are functionally interrelated, or they interact with the TCR$\alpha\beta$, TCR$\gamma\delta$, or putative NK lymphocyte receptors and with each other. As we originally proposed [17], such synergism has now been determined between, e.g. CD2 and CD3, and has also been identified in MHC-restricted antigen recognition [18]. The functional interplay between distinct activation sites suggests that their individual activation pathways converge intracellularly [17,19–21]. These data strongly argue in favor of the hypothesis that a multireceptor recognition process is involved in MHC-unrestricted target-cell recognition and lysis [22,23]. Therefore, activation

via one receptor can be blocked via another using the relevant mAb [13,17,19,20,24–27].

ENGINEERING OF LYMPHOCYTE SPECIFICITY

Because most efforts to produce human CTL specific for human tumors have failed and because many mAb are available that bind selectivity, although not exclusively, to tumor cells, the combined use of CTL and mAb, offered a novel approach to selectively direct the CTL to the tumor cells. These engineered lymphocyte specificities may in the future play a key role in the destruction of tumor or virus-infected cells. The employment of mAb, bs-mAb or chimeric TCR-Ig receptors to engineer lymphocyte specificity together with cytokines represents a promising immunotherapeutic approach.

Monoclonal Antibody Directed Lymphocyte Mediated Lysis

After the discovery that an anti-clonotypic mAb producing hybridoma was lysed by the CTL specifically binding their mAb [28] it was reported that lysis could also be induced using mAb specific for the nonpolymorphic CD3 by mAb against CD2, expressed by T and NK lymphocytes; mAb against CD16, expressed on virtually all NK lymphocytes and a subset of TCR$\gamma\delta$ lymphocytes, or mAb against $CD26^+$ T-lymphocytes. Noteworthy, induction of cytolysis only occurred when the target cells expressed receptors, that bind the Fc-parts of the mAb, i.e. IgG-FcR (CD16-FcR) [29].

Bispecific Monoclonal Antibody Targeting of CTL

Thus, an interesting exploitation of the phenomenon that mAb mediated lymphocyte activation results in triggering of the lytic machinery (see above) is the use of bs-mAb [30,31]. Bs-mAb can be produced by chemically coupling a mAb that is specific for the TCR-complex, for example to a second mAb specific for a given tumor associated antigen (TAA) structure. For instance, chemically crosslinked antibodies, one binding site recognizing the CD16 activation site on the effector lymphocytes, and the other recognizing a TAA [32], have been produced. Such mAb heteroaggregates bridge the effector lymphocyte to the target cell (conjugate formation) and trigger the lymphocyte lytic machinery. The heteroconjugated antibody triggers cytolysis by binding to CD16 via its Fab, rather than via its Fc-portion. Along the same line, many preparations of bs-mAb, that recognize the CD3 complex on the one hand and a TAA on the other (e.g. melanoma, renal, ovarian, lung and mammary carcinoma) have been developed [33–37]. Such bs-mAb have also been generated by fusion of two hybridomas that produce CD3-specific mAb and the target-cell-specific mAb respectively [38]. The hallmark of this mechanism is that the binding of the bs-mAb to the CTL endows the CTL with the bs-mAb specificity. These bs-mAb targeted CTL can effectively be used for adoptive transfer of immunity [39].

Since mAb with specificity for particular lymphocyte activation sites trigger different functions or lymphocyte subpopulations, their use also allows the selective activation and expansion of particular subsets of lymphocytes and eliminates the need for prior isolation of these subsets.

These laboratory-engineered immune lymphocytes are presently being used for clinical adoptive immunotherapy. We and others have recently started a phase I-II clinical trial employing bs-mAb retargeted lymphocytes for the intraperitoneal treatment of ovarian carcinoma patients [40].

Chimeric T Cell Receptor-Immunoglobulin

Bs-mAb targeted T lymphocytes retain the antibody-dictated specificity for limited periods of time (6–72 h) due to the dissociation of the bs-mAb from the cell surface [33, and Bolhuis, manuscript in preparation]. In addition, it is not known whether or not the mAb targeted lymphocytes can traffic and home to the tumor site. Bs-mAb targeted T lymphocytes are therefore expected to be clinically most effective when applied locally for the cytolytic attack and the initiation of inflammatory reactions. The total eradication of established tumors requires a "long-lasting" systemic immune response. Recently, molecular engineering of T lymphocytes has resulted in permanently acquired, laboratory chosen, antibody-dictated specificity of lymphocytes. To become effective, such T lymphocytes require not only the stable expression of the engineered receptor at the lymphocyte surface, but also an association of this receptor with their signal-transducing elements. These requirements were met by the introduction and expression of chimeric TCR/Ig genes, in which

the variable (V) gene segments of the TCRα and TCRβ chains were replaced by the variable gene segments of the heavy or light chain of an Ig with known specificity, e.g., against phosphatidylcholine, trinitrophenyl or digoxin [41–47]. Upon transfection of the expression vectors containing these chimeric TCR/Ig genes in recipient mouse T cell lines, chimeric proteins were synthesized and became stably and functionally expressed at the surface of the lymphocytes in association with the signal transducing CD3 complex. Both combinations of complementary chimeric TCR/Ig genes, i.e., V_H-Cα + V_L-Cβ or V_H-Cβ + V_L-Cα, produced a functional chimeric heterodimer [42,46]. Consequently, the chimeric receptor endowed the T lymphocytes with the antibody-dictated, MHC-unrestricted specificity, and was able to transduce signals for T cell activation as determined by Ca_i^{2+} mobilization [46]; IL-2 production [41–44,47]; proliferative response [47], and cytotoxic activity [42,43,47].

In view of anticipated clinical applications it is important that a high percentage of lymphocytes can be simultaneously infected. This will reduce the need of prolonged lymphocyte cultures in order to yield the large number of lymphocytes required for immunotherapy today. In addition, it ensures the polyclonality of the genetically modified T lymphocytes. In our opinion this can be best achieved by retroviral mediated gene transfer, which can transfect a wide variety of cell types with a much higher efficiency than other procedures [48].

Indeed, mouse and human T lymphocytes have been successfully transduced with various genes using retroviral vectors [48–53]. An additional advantage of retrovirus-mediated gene transfer is that all proviral copies become stably integrated into the chromosomal DNA of the recipient cell. This chromosomal integration of the transduced gene ensures the constitutive expression of chimeric TCR/Ig genes, a prerequisite for prolonged immune reactivity.

Before clinical application of these genetically modified lymphocytes becomes a reality, the safety of the administration of these lymphocytes has to be assessed, because foreign DNA is inserted randomly in the genome. This may theoretically trigger an oncogenic transformation. So far, studies, in vitro in animals and 56 months of observation of patients who received neo gene transfected autologous lymphocytes in a phase I clinical protocol have not revealed any abnormalities or side-effects.

The results of these novel strategies are therefore crucial in aiding in the development of novel immunotherapeutic strategies and will also advance our understanding of lymphocyte receptors mediated Ag recognition.

REFERENCES

1. Ehrlich P: Ned Tijdschr Geneeskd 35:273–290, 1909.
2. Burnet FM: Prog Exp Tum Res 13:1–27, 1970.
3. Bubenik J, Jakoubkova J, Krakora P, Baresova M, Helbich P, Vicklicky V, Malaskova V: Int J Cancer 8:503–513, 1971.
4. Vose BM, White W: Cancer Immunol Immunother 15: 227–236, 1983.
5. Uchida A, Kariya Y, Okamoto N, Sugie K, Fujimoto T, Yagita M: J Natl Cancer Inst 82:1697–1701, 1990.
6. Bolhuis RLH, Gravekamp C, Van de Griend RJ: Clinics in Immunology and Allergy 6:29–90, 1986.
7. Davis MM, Bjorkman PJ: Nature 334:395–402, 1988.
8. Trinchieri G: Adv Immunol 47:187–376, 1989.
9. Ortaldo JR, Kantor R, Segal D, Bolhuis RLH, Bino T: Natural Killer Cells and Host Defense 37:221–226, 1989.
10. Brenner MB, McLean J, Dialynas DPB, Strominger JL, Smith JA, Owen FL, Seidman JG, Ip S, Rosen F, Krangel MS: Nature 322:145–149, 1986.
11. Borst J, Van de Griend RJ, Van Oostveen H, Ang S, Melief CJM, Seidman JG, Bolhuis RLH: Nature 325: 683–688, 1987.
12. Brenner MB, McLean J, Scheft H, Riberdy J, Ang SL, Seidman SG, Devlin P, Krangel MS: Nature 325:689–694, 1987.
13. Sturm E, Braakman E, Bontrop RE, Chuchana P, Van de Griend RJ, Koning F, Lefranc MP, Bolhuis RLH: Eur J Immunol 19:1261–1265, 1989.
14. Spits H, Yssel H, de Vries JE: Res Immunol 141:636–640, 1990.
15. Acuto O, Hussey RE, Fitzgerald KA, Protentis JP, Meuer SC, Schlossman SF, Reinherz EL: Cell 34:717–726, 1983.
16. Staertz UD, Pasternack MS, Klein JR, Benedetto JD, Bevan MJ: Proc Natl Acad Sci USA 1799–1803, 1984.
17. Bolhuis RLH, Roozemond RC, Van de Griend RJ: J Immunol 136:3939–3944, 1986.
18. Moingeon P, Chang HC, Wallner BP, Stebbins C, Frey AZ, Reinherz EL: Nature 339:312–314, 1989.
19. Pantaleo G, Olive D, Poggi A, Pozzan T, Moretta L, Moretta A: J Exp Med 166:619–624, 1987.
20. Van de Griend RJ, Bolhuis RLH, Stoter G, Roozemond RC: J Immunol 138:3137–3144, 1987.
21. Alcover A, Albertini C, Acuto O, Clayton LK, Transy C, Spagnoli GC, Moingeon P, Lopez P, Reinherz EL: EMBO J 7:1973–1977, 1988.
22. Hersey P, Bolhuis RLH: Immunol Today 8:233–239, 1987.
23. Bolhuis RLH, Braakman E: Curr Opin Immunol 1:236–240, 1988.
24. Braakman E, Goedegebuure PS, Vreugdenhil RJ, Segal DM, Shaw S, Bolhuis RLH: Int J Cancer 46:475–480, 1990.

25. Goedegebuure PS, Braakman E, Segal DM, Vreugdenhil RJ, Bolhuis RLH: Int Immunol 2:1213–1220, 1990.
26. Bolhuis RLH, Van de Griend RJ: Cellular Immunol 93:46–57, 1985.
27. Spits H, Yssel H, Voordouw A, de Vries JE: J Immunol 134:2294–2298, 1985.
28. Lancki DW, Ma DI, Havran WL, Fitch FW: Immunol Rev 81:65–94, 1984.
29. Davies DR, Sheriff S, Padlan EA: J Biol Chem 263: 10541–10544, 1988.
30. Staertz UD, Kanagawa O, Bevan MJ: Nature 314:628–631, 1985.
31. Perez P, Hoffman RW, Shaw S, Bluestone JA, Segal DM: Nature 316:354–356, 1985.
32. Karpovsky B, Titus JA, Stephany DA, Segal DM: J Exp Med 160:1686–1701, 1984.
33. Lanzavecchia A, Scheidegger D: Eur J Immunol 17:105–111, 1987.
34. Titus JA, Garrido MA, Hecht TT, Winkler DF, Wunderlich JR, Segal DM: J Immunol 138:4018–4022, 1987.
35. Canevari S, Ménard S, Mezzanzanica D, Miotti S, Pupa SM, Lanzavecchia A, Colnaghi MI: Int J Cancer 2:18–21, 1988.
36. Van Dijk J, Tsuruo T, Segal DM, Bolhuis RLH, Colognola R, Van de Griend RJ, Fleuren GJ, Warnaar SO: Int J Cancer 44:738–743, 1989.
37. Van Dijk J, Warnaar SO, Van Eendenburg JDH, Thienpont M, Braakman E, Boot JHA, Fleuren GJ, Bolhuis RLH: Int J Cancer 43:344–349, 1989.
38. Milstein C, Cuello AC: Nature 305:537–540, 1983.
39. Segal DM, Snider DP: Chem Immunol 47:179–213, 1989.
40. Nitta T, Sato K, Yagita H, Okumura K, Ishii S: Lancet 335:368–371, 1990.
41. Eshhar Z, Gross G: Br J Cancer 62(suppl) 10:27–29, 1990.
42. Gross G, Waks T, Eshhar Z: Proc Natl Acad Sci USA 86:10024–10028, 1989.
43. Gross G, Gorochov G, Waks T, Eshhar Z: Transplant Proc 21:127–130, 1989.
44. Goverman J, Gomez SM, Segesman KD, Hunkapiller T, Lang WE, Hood L: Cell 60:929–939, 1990.
45. Becker MLB, Near R, Mudgett-Hunter M, Margolies MN, Kubo RT, Kaye J, Hedrick SM: Cell 58:911–921, 1989.
46. Kuwana Y, Asakura Y, Utsuomiya N, Nakanishi M, Arata Y, Itoh S, Nagase F, Kurosawa Y: Biochem Biophys Res Commun 149:960–968, 1987.
47. Gorochov G, Gross G, Waks T, Eshhar Z: Cellular immunity and the immunotherapy of cancer. In Lotze MT, Finn OJ (Eds): "UCLA Symposia on Molecular and Cellular Biology, New Series" New York Wiley-Liss Inc. pp 45–51, 1990.
48. Cepko CL, Roberts BE, Mulligan RC: Cell 37:1053–1062, 1984.
49. Kasid A, Morecki S, Aebersold P, Cornetta K, Culver K, Freeman S, Director E, Lotze MT, Blaese RM, Anderson WF, Rosenberg SA: Proc Natl Acad Sci USA 87:473–477, 1990.
50. Rosenberg SA, Aebersold P, Cornetta K, Kasid A, Morgan RA, Moen R, Karson EM, Lotze MT, Yang JC, Topalian SL, Merino MJ, Culver K, Miller AD, Blaese RM, Anderson WF: N Engl J Med 323:570–578, 1990.
51. Finn OJ, Persons DA, Bendt KM, Pirami L, Ricciardi P: J Immunol 146:1099–1103, 1991.
52. Abraham N, Miceli M, Parnes JR, Veilette A: Nature 350:62–66, 1991.
53. Ferrari G, Rossini S, Giavazzi R, Maggioni D, Nobili N, Soldati M, Ungers G, Mavilio F, Gilboa E, Bordignon C: Science 251:1363–1366, 1991.