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EDITORIAL

The strange case of TGN1412

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A current focus of cancer research is the development of immunostimulatory therapeutic antibodies, based on the notion that increasing the number or activity of cytotoxic T lymphocytes, and in particular those directed against tumour-associated antigens, may achieve effective immune mediated tumour rejection. Unlike other techniques, such as the use of antibodies specific to tumour cells themselves, this approach does not rely on antigen-specificity, and accordingly is subject to risks of autoimmunity as a consequence of non-specific activation of T cells [1].

The risks associated with such an approach have been placed firmly in the spotlight following the recent highly publicised suspension of Phase-I clinical testing of TGN1412 (see [2] for TGN1412 Investigator's Brochure). This monoclonal antibody, one of a class of 'superagonists' for the immune co-stimulatory receptor CD28, was developed by TeGenero Immune Therapeutics (Würzburg, Germany) for the treatment of both autoimmune diseases [3] and leukaemia [4, 5]. Use of such antibodies for treatment of both autoimmunity and malignancy seeks to exploit the opposing functions of the antibody; in the former, the aim is the expansion of regulatory T cells for the suppression of self-directed immune responses, whereas in the latter, the aim is expansion and activation of cytotoxic T cells directed against tumour antigens. Shortly after being injected with the TGN1412, all six healthy volunteers were admitted into intensive care with rapid onset grade-IV toxicity, suffering from multi-organ failure, caused by cytokine release syndrome [6].

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One man's agonist...

The natural ligands for CD28 are the B7 family of antigens (B7.1/CD80 and B7.2/CD86), found on antigen presenting cells (APCs) [7]. Under natural conditions, T cell activation requires two separate stimulatory signals: (1) T cell receptor (TCR) engagement of antigen presented by MHC, and (2) binding of co-stimulatory molecules such as B7 to its cognate receptor CD28 [8, 9] (Fig. 1a). Binding of B7 to CD28 causes expansion, proliferation and cytokine release by T cells [10, 11]. However, if either signal is provided with sufficient intensity, as for instance by superantigen stimulation of TCR (Fig. 1b), or antibody mediated super activation of CD28 (Fig. 1c), T cell activation can be achieved without the need for the second signal [12–16]. Therefore, TGN1412 mediated activation of CD28 should be able to induce antigen independent, polyclonal, T cell activation. Indeed, in pre-clinical studies for the treatment of B-cell chronic lymphocytic leukaemia, TGN1412 has been reported to stimulate polyclonal T cell activation [4, 5].

However, during the development of TGN1412, a number of studies were published reporting that superagonistic anti-CD28 antibodies could induce the preferential activation of $CD4^+CD25^+$ T cells (T_{regs}), rather than polyclonal activation of conventional

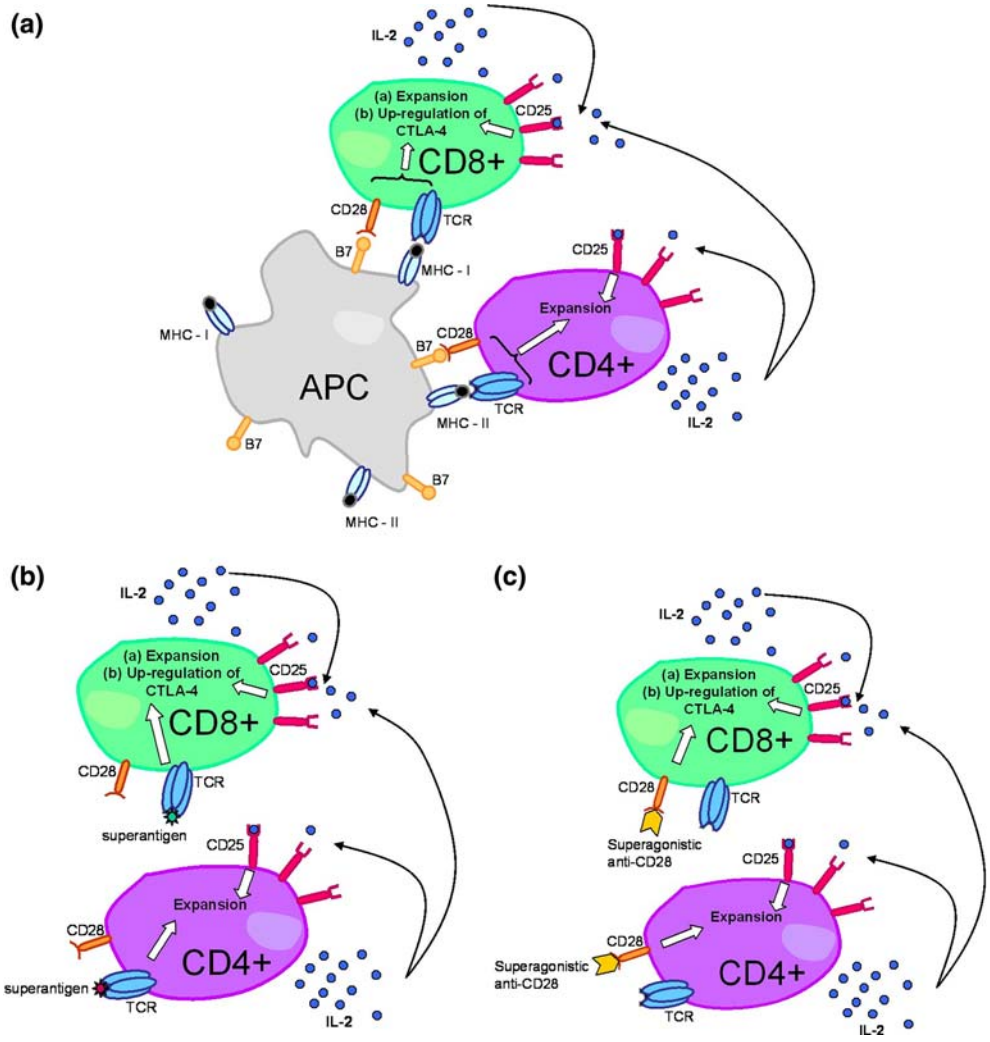

Fig. 1 APC-mediated activation of CD4 and CD8 T cells involves MHC/antigen-mediated stimulation of TCR, as well as the activation of co-stimulatory signalling pathways (**a**). However, potent stimulation of TCR (e.g. by superantigens) (**b**) or CD28 (e.g. by TGN1412) (**c**) can bypasses the need for the second signal

T cells [3, 17–19]. T_{regs} suppress the activation of T cells, including autoreactive T cells that have remained in the mature T cell population by evading thymic elimination and/or interaction with tolerising APCs [1, 20–22]. Therefore, preferential expansion of T_{regs} would result in active suppression of autoreactive T cells. In contrast, the polyclonal expansion of conventional T cells would cause the activation of autoreactive T cells and induction of autoimmune responses [23, 24].

Other studies have independently established that expansion of T_{regs} is highly dependent on the activation of CD28 [25, 26]. Nonetheless, the preferential expansion of T_{regs} by antibody mediated activation of CD28 is extremely dose-dependent; in one study employing an experimental model of autoimmune encephalomyelitis, a dose of 0.5 or 1.5 mg/kg elicited preferential expansion of T_{regs} [27]. However, this preferential expansion of T_{regs} was no longer detectable at the higher dose of 5 mg/kg [27].

A similar example of dose sensitivity has been illustrated in clinical trials of recombinant IL-2/diphtheria toxin conjugate DAB_{389}IL-2 (also known as denileukin diftitox or ONTAK) for the vaccination mediated immune therapy of metastatic renal cell carcinoma patients. Whilst this IL-2 conjugate preferentially eliminated T_{regs}, the effects were highly dose dependent and transient [28]. Thus, common molecular targets that can be used both for preferential expansion of T_{regs} and for polyclonal expansion of conventional T cells can be expected to be highly dose sensitive, resulting in dramatically different outcomes at different doses.

Finding the right dose

The recent anti-CD28 trial has also served to highlight the problems that can arise when designing animal safety studies to test a humanised antibody. Herein lies a conundrum; if a non-humanised antibody is used in animal safety studies, it might not betray potential toxic effects that may arise as a consequence of humanisation of the antibody. Therefore, such a safety study would be incomplete and uninformative of the corresponding human dose. By contrast, if a humanised antibody is used in animal studies, it may be neutralised swiftly, thus masking the harmful side effects of the drug, making it a challenging task to properly estimate the appropriate dose range for human use. This is an important concern in the case of monoclonal antibodies, as they are likely to be highly dose dependent and species specific [29]. This conflict may be partially resolved by the development of transgenic animal models, able to express human orthologues of the relevant genes, providing a more realistic model for testing of humanised antibodies [30].

Moreover, given the documented possible autoimmune side effects of this class of therapies, and the fact that they are designed for administration to patients with altered immune status (cancer or autoimmunity), the wisdom of using healthy volunteers in the TGN1412 trial is questionable. However, whilst patients may have proved to be a more suitable choice, the limits of feasibility and difficulties in recruitment of suitable patients may restrict this choice in practice.

Lessons from CTLA-4

Similarly to CD28, the cell surface protein CTLA-4 also binds B7. In contrast to the action of CD28, CTLA-4 engagement of B7 actively inhibits T cell stimulation, inducing apoptosis or anergy in the activated T cells (Fig. 2a) [31–34]. Although it is presented on the T cell surface at a lower density than CD28, CTLA-4 has a ~20-fold higher affinity for the B7 ligand [35]. Activation of naive T cells results in increased cell surface expression of CTLA-4 [36], thus creating a negative feedback mechanism to moderate their activation. CTLA-4 is constitutively expressed on the surface of $CD4^+CD25^+$ T_{regs} [37, 38]. Therefore, CD28 and CTLA-4 play antagonistic roles in the regulation of T cells [39, 40], maintaining a fine balance between CD28-mediated activation and CTLA-4-mediated inhibition of T cell activation.

Although it is difficult to detect CTLA-4 expression on resting T cells, in stimulated cells it is rapidly trafficked to the cell surface, whereupon CTLA-4 ligation effectively blocks even the earliest events in T-cell activation, including cytokine production and cell proliferation [32, 41]. The key regulatory role of CTLA-4 in 'dampening' of an immune response is demonstrated clearly in CTLA-4 knockout mice, which suffer from fatal organ destruction as a result of lymphoproliferation [42, 43]. Moreover, mutations in CTLA-4 in humans are implicated in several autoimmune diseases such as type-I diabetes [44]. Inhibitory antibody mediated blockade of CTLA-4 (Fig. 2b) in murine models is effective in reducing tumour size, but these models also show autoimmune side effects ranging from loss of skin pigmentation (vitiligo) to allergic encephalomyelitis [45, 46]. Autoimmune side effects have also been documented in

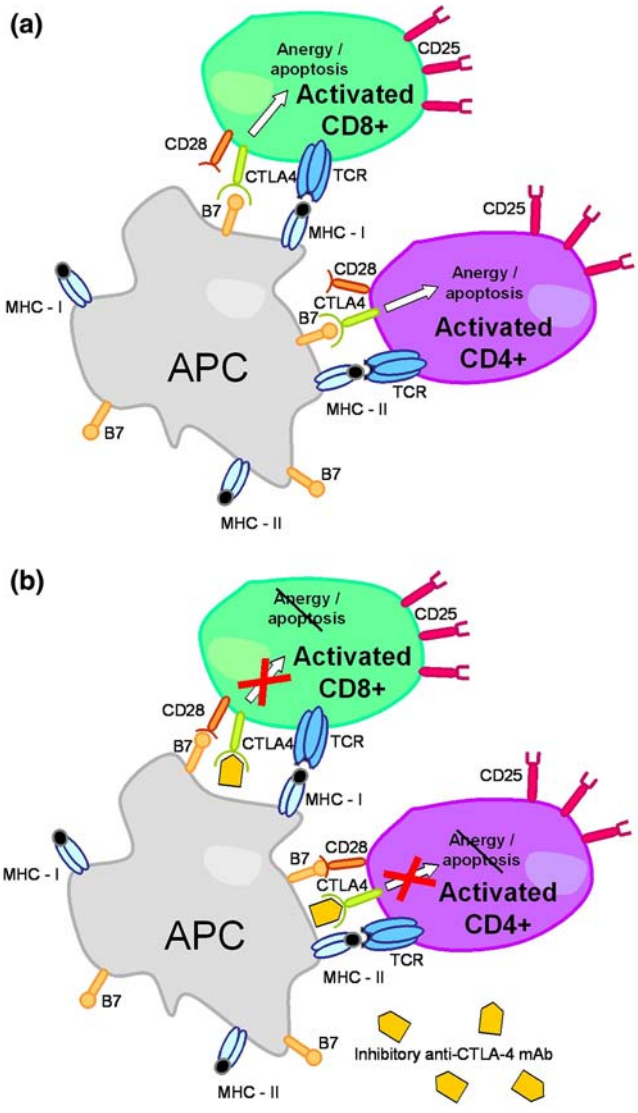

Fig. 2 B7 ligation of CTLA-4 (**a**) triggers apoptosis or energy in T cell populations. Inhibition of CTLA-4 signalling (**b**) prevents apoptosis/anergy in the activated T cell population

human clinical trials of an inhibitory anti-CTLA-4 antibody in a number of different human cancers [47–50].

Anti-CTLA-4 trials thus provide an important point of comparison with the anti-CD28 therapeutics. Despite the fact that CTLA-4 is expressed constitutively on $CD4^+$ $CD25^+$ T_{regs}, there is experimental data to suggest that CTLA-4 blockade also promotes an increase in the levels of conventional T cells [51]. Hence, both immunosuppressive and enhanced autoimmune effects should be considered as possible sequelae of CTLA-4 blockade therapy. Arguably, there is no currently available technology for the reliable preferential expansion of T_{regs} over conventional T cells.

It should be noted that anti-CTLA-4 therapies only target activated T cells, due to the absence of CTLA-4 on the surface of naive T cells. By contrast, superagonistic anti-CD28 antibodies can act upon both naive and antigen-experienced T cells [52, 53], and therefore have the potential for a much more powerful and indiscriminate immune response.

Tethering the beast

The magnitude and rapidity of the response observed in the TGN1412 trial does not appear to be compatible with the activation of antigen-specific cytotoxic T lymphocytes, as this would not produce observable side effects until much later. The swift and severe side effects exhibited in the TGN1412 trial are much more consistent with the rapid release of a variety of pro-inflammatory cytokines – known as a "cytokine storm" [12], possibly due to CD28 mediated polyclonal stimulation of T cells. Interestingly, a recent study examining superantigen-mediated toxic shock, in which similar rapid responses are observed, has reported a biphasic pattern of cytokine response, with an early rise in TNF-α suggested to be responsible for swift manifestation of symptoms, rather than the later cytokine surge [16]. Certainly, the clinical situation is likely to be much more complex, and a previous study of cytokine storm-related sequelae following systemic IL-2 administration for renal cell carcinoma has indeed reported both quantitative and qualitative variations in the pattern of cytokine expression among individual patients [54]. Nonetheless, the potent responses manifested in all six previously healthy volunteers receiving TGN1412 suggest the promiscuous activation of an overriding mechanism.

Both anti-CTLA-4 and anti-CD28 therapies rely on releasing free antibody into the body, exacerbating the risk of a runaway immune response, since the kinetics of the interaction are pushed in favour of widespread expansion. By contrast, strategies which aim to modulate antigen specific T cells, for instance tumour cell vaccines generated by gene transfer mediated expression of B7.1 (CD80) on the surface of tumour cells, are distinct in that they have a tethered co-stimulator, as opposed to soluble, free, antibody [55–57].

While polyclonal expansion of T cells is observed in B7.1 over-expressing transgenic mice [58], B7.1 on the surface of tumour cells can activate only the T cell subsets that are able to engage the MHC-antigen on these cells. This allows a more selective stimulation of T cells that are able to access the B7 expressing tumour cell vaccine, whilst retaining the CTLA-4 mediated negative feedback loop for prevention of run-away T cell activation.

Concluding remarks

Despite the tremendous therapeutic potential of antibody-mediated T cell regulation, the recent TGN1412 trial has highlighted a number of key issues surrounding design of clinical trials and potential risks associated with the manipulation of broad ranging immunological responses. There are of course a host of other potential factors that may have caused the severe side effects in the TGN1412 trial, including manufacturing and/or dosing errors. However, it appears that there is an all too fine balance between boosting an immune response, and triggering autoimmunity. Given the inherent sensitivity of immune therapy based strategies, every care must be taken to increase the safety of such clinical trials. On a practical level, this entails using recruitment and dosing schedules to allow the assessment of at least the most severe/acute side effects in individual patients before proceeding with larger patient cohorts. In addition, careful risk/benefit analyses should feature centrally in the choice between the use of healthy versus patient volunteers. The TGN1412 trial has also highlighted the need for detailed attention to the relevance of pre-clinical animal studies, particularly with reference to both the dose and specificity of humanised antibodies. Development of safe and effective means of manipulation of regulatory T cells for therapeutic applications remains an important target in drug development. Only once the selective manipulation of regulatory T cells can be consistently achieved in vivo, can the full potential of this technology be finally realised.

References

1. Gilboa E (2001) The risk of autoimmunity associated with tumor immunotherapy. Nat Immunol 2:789–792

2. TGN1412 Investigator's Brochure. http://www.mhra.gov.uk/home/groups/es-foi/documents/foidisclosure/con2023525.pdf
3. Beyersdorf N, Hanke T, Kerkau T, Hunig T (2005) Superagonistic anti-CD28 antibodies: potent activators of regulatory T cells for the therapy of autoimmune diseases. Ann Rheum Dis 64:91–95
4. Luhder F, Huang Y, Dennehy KM, Guntermann C, Muller I, Winkler E, Kerkau T, Ikemizu S, Davis SJ, Hanke T, Hunig T (2003) Topological requirements and signaling properties of T cell-activating, anti-CD28 antibody superagonists. J Exp Med 197:949–953
5. Lin C-H, Kerkau T, Guntermann C, Trischler M, Beyersdorf N, Scheuring Y, Tony H-P, Kneitz C, Wilhelm M, Mueller P, Huenig T, Hanke T (2004) Superagonistic anti-CD28 antibody TGN1412 as a potential immunotherapeutic for the treatment of B cell chronic lymphocytic leukemia. Blood 104:690A
6. MHRA Press release (2006) Investigations into adverse incidents during clinical trials of TGN1412: interim report. http://www.mhra.gov.uk/home/groups/comms-po/documents/websiteresources/con2023519.pdf
7. Linsley PS, Clark EA, Ledbetter JA (1990) T-cell antigen CD28 mediates adhesion with B-cells by interacting with activation antigen B7/B7-1. Proc Natl Acad Sci USA 87:5031–5035
8. Gimmi CD, Freeman GJ, Gribben JG, Sugita K, Freedman AS, Morimoto C, Nadler LM (1991) B-cell surface antigen-B7 provides a costimulatory signal that induces T-cells to proliferate and secrete interleukin-2. Proc Natl Acad Sci USA 88:6575–6579
9. Lenschow DJ, Walunas TL, Bluestone JA (1996) CD28/B7 system of T cell costimulation. Annu Rev Immunol 14:233–258
10. Koulova L, Clark EA, Shu G, Dupont B (1991) The CD28 ligand B7/BB1 provides costimulatory signal for alloactivation of $CD4^+$ T-cells. J Exp Med 173:759–762
11. Linsley PS, Brady W, Grosmaire L, Aruffo A, Damle NK, Ledbetter JA (1991) Binding of the B-cell activation antigen B7 to CD28 costimulates T-cell proliferation and interleukin-2 messenger-RNA accumulation. J Exp Med 173:721–730
12. Bette M, Schafer MKH, Van Rooijen N, Weihe E, Fleischer B (1993) Distribution and kinetics of superantigen-induced cytokine gene-expression in mouse spleen. J Exp Med 178:1531–1540
13. Brinkmann V, Kinzel B, Kristofic C (1996) TCR-independent activation of human $CD4^{(+)}45RO^{(-)}$ T cells by anti-CD28 plus IL-2 – induction of clonal expansion and priming for a Th2 phenotype. J Immunol 156:4100–4106
14. Tacke M, Hanke G, Hanke T, Hunig T (1997) CD28-mediated induction of proliferation in resting T cells in vitro and in vivo without engagement of the T cell receptor: evidence for functionally distinct forms of CD28. Eur J Immunol 27:239–247
15. Raab M, Pfister S, Rudd CE (2001) CD28 signaling via VAV/SLP-76 adaptors: regulation of cytokine transcription independent of TCR ligation. Immunity 15:921–933
16. Faulkner L, Cooper A, Fantino C, Altmann DM, Sriskandan S (2005) The mechanism of superantigen-mediated toxic shock: not a simple Th1 cytokine storm. J Immunol 175:6870–6877
17. Lin CH, Hunig T (2003) Efficient expansion of regulatory T cells in vitro and in vivo with a CD28 superagonist. Eur J Immunol 33:626–638
18. Beyersdorf N, Hanke T, Kerkau T, Hunig T (2006) CD28 superagonists put a break on autoimmunity by preferentially activating $CD4^{(+)}CD25^{(+)}$ regulatory T cells. Autoimmun Rev 5:40–45
19. Hunig T, Dennehy K (2005) CD28 superagonists: mode of action and therapeutic potential. Immunol Lett 100:21–28
20. Bluestone JA, Tang Q (2005) How do $CD4^+CD25^+$ regulatory T cells control autoimmunity? Curr Opin Immunol 17:638–642
21. Wei W-Z, Morris GP, Kong Y-CM (2004) Anti-tumour immunity and autoimmunity: a balancing act of regulatory T cells. Cancer Immunol Immunother 53:73–78
22. Chattopadhyay S, Chakraborty NG, Mukherji B (2005) Regulatory T cells and tumour immunity. Cancer Immunol Immunother 54:1153–1161
23. Chatila TA (2005) Role of regulatory T cells in human diseases. J Allergy Clin Immunol 116:949–959
24. Hsieh CS, Liang Y, Tyznik AJ, Self SG, Liggitt D, Rudensky AY (2004) Recognition of the peripheral self by naturally arising $CD25^{(+)}$ $CD4^{(+)}$ T cell receptors. Immunity 21:267–277
25. Tang Q, Henriksen KJ, Boden EK, Tooley AJ, Ye J, Subudhi SK, Zheng XX, Strom TB, Bluestone JA (2003) Cutting edge: CD28 controls peripheral homeostasis of $CD4^{(+)}CD25^{(+)}$. J Immunol 171:3348–3352
26. Schmidt J, Elflein K, Stienekemeier M, Rodriguez-Palmero M, Schneider C, Toyka KV, Gold R, Hunig T (2003) Treatment and prevention of experimental autoimmune neuritis with superagonistic CD28-specific monoclonal antibodies. J Neuroimmunol 140:143–152
27. Beyersdorf N, Gaupp S, Balbach K, Schmidt J, Toyka KV, Lin CH, Hanke T, Hunig T, Kerkau T, Gold R (2005) Selective targeting of regulatory T cells with CD28 superagonists allows effective therapy of experimental autoimmune encephalomyelitis. J Exp Med 202:445–455
28. Dannull J, Su Z, Rizzieri D, Yang BK, Coleman D, Yancey D, Zhang A, Dahm P, Chao N, Gilboa E, Vieweg J (2005) Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. J Clin Invest 115:3623–3633
29. Weinberg WC, Frazier-Jessen MR, Wu WJ, Weir A, Hartsough M, Keegan P, Fuchs C (2005) Development and regulation of monoclonal antibody products: challenges and opportunities. Cancer Metastasis Rev 24:569–584
30. Pascolo S (2005) HLA class I transgenic mice: development, utilisation and improvement. Expert Opin Biol Ther 5:919–938
31. Alegre ML, Frauwirth KA, Thompson CB (2001) T-cell regulation by CD28 and CTLA-4. Nat Rev Immunol 1:220–228
32. Brunner MC, Chambers CA, Chan FK, Hanke J, Winoto A, Allison JP (1999) CTLA-4-mediated inhibition of early events of T cell proliferation. J Immunol 162:5813–5820
33. Gribben JG, Freeman GJ, Boussiotis VA, Rennertt P, Jellist CL, Greenfieldt E, Barber M, Restivo VA, Ke X, Grayt GS, Nadler LM (1995) CTLA4 mediates antigen-specific apoptosis of human T-cells. Proc Nat Acad Sci USA 92:811–815
34. Walunas TL, Bakker CY, Bluestone JA (1996) CTLA-4 ligation blocks CD28-dependent T cell activation. J Exp Med 183:2541–2550
35. Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA (1991) CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med 174:561–569
36. Krummel MF, Allison JP (1996) CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. J Exp Med 183:2533–2540
37. Alegre ML, Shiels H, Thompson CB, Gajewski TF (1998) Expression and function of CTLA-4 in Th1 and Th2 cells. J Immunol 161:3347–3356
38. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, Mak TW, Sakaguchi S (2000) Immunologic self-tolerance maintained by $CD25^{(+)}CD4^{(+)}$ regulatory T cells

constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 192:303–310
39. Allison JP, Krummel MF (1995) The Yin and Yang of T-Cell costimulation. Science 270:932–933
40. Krummel MF, Sullivan TJ, Allison JP (1996) Superantigen responses and co-stimulation: CD28 and CTLA-4 have opposing effects on T cell expansion in vitro and in vivo. Int Immunol 8:519–523
41. Blair PJ, Riley JL, Levine BL, Lee KP, Craighead N, Francomano T, Perfetto SJ, Gray GS, Carreno BM, June CH (1998) CTLA-4 ligation delivers a unique signal to resting human CD4 T cells that inhibits interleukin-2 secretion but allows Bcl-X(L) induction. J Immunol 160:12–15
42. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH (1995) Loss of Ctla-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 3:541–547
43. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, Thompson CB, Griesser H, Mak TW (1995) Lymphoproliferative disorders with early lethality in mice deficient in CTLA-4. Science 270:985–988
44. Donner H, Braun J, Seidl C, Rau H, Finke R, Ventz M, Walfish PG, Usadel KH, Badenhoop K (1997) CTLA4 alanine-17 confers genetic susceptibility to Graves' disease and to type 1 diabetes mellitus. J Clin Endocrinol Metab 82:143–146
45. Hurwitz AA, SullivanTJ, Krummel MF, Sobel RA, Allison JP (1997) Specific blockade of CTLA-4/B7 interactions results in exacerbated clinical and histologic disease in an actively-induced model of experimental allergic encephalomyelitis. J Neuroimmunol 73:57–62
46. van Elsas A, Hurwitz AA, Allison JP (1999) Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. J Exp Med 190:355–366
47. Phan GQ, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, Restifo NP, Haworth LR, Seipp CA, Freezer LJ, Morton KE, Mavroukakis SA, Duray PH, Steinberg SM, Allison JP, Davis TA, Rosenberg SA (2003) Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci USA 100:8372–8377
48. Sanderson K, Scotland R, Lee P, Liu D, Groshen S, Snively J, Sian S, Nichol G, Davis T, Keler T, Yellin M, Weber J (2005) Autoimmunity in a phase I trial of a fully human anti-cytotoxic T-lymphocyte antigen-4 monoclonal antibody with multiple melanoma peptides and montanide ISA 51 for patients with resected stages III and IV melanoma. J Clin Oncol 23:741–750
49. Maker AV, Phan GQ, Attia P, Yang JC, Sherry RM, Topalian SL, Kammula US, Royal RE, Haworth LR, Levy C, Kleiner D, Mavroukakis SA, Yellin M, Rosenberg SA (2005) Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte-associated antigen 4 blockade and interleukin 2: a phase I/II study. Ann Surg Oncol 12:1005–1016
50. Blansfield JA, Beck KE, Tran K, Yang JC, Hughes MS, Kammula US, Royal RE, Topalian SL, Haworth LR, Levy C, Rosenberg SA, Sherry RM (2005) Cytotoxic T-Lymphocyte-associated antigen-4 blockage can induce autoimmune hypophysitis in patients with metastatic melanoma and renal cancer. J Immunother 28:593–598
51. Maker AV, Attia P, Rosenberg SA (2005) Analysis of the cellular mechanism of antitumor responses and autoimmunity in patients treated with CTLA-4 blockade. J Immunol 175:7746–7754
52. Riley JL, Mao M, Kobayashi S, Biery M, Burchard J, Cavet G, Gregson BP, June CH, Linsley PS (2002). Modulation of TCR-induced transcriptional profiles by ligation of CD28, ICOS, and CTLA-4 receptors. Proc Natl Acad Sci USA 99:11790–11795
53. Diehn M, Alizadeh AA, Rando OJ, Liu CL, Stankunas K, Botstein D, Crabtree GR, Brown PO (2002) Genomic expression programs and the integration of the CD28 costimulatory signal in T cell activation. Proc Natl Acad Sci USA 99:11796–11801
54. Panelli MC, White R, Foster M, Martin B, Wang E, Smith K, Marincola FM (2004). Forecasting the cytokine storm following systemic interleukin (IL)-2 administration. J Transl Med 2:17
55. Chan L, Hardwick N, Darling D, Galea-Lauri J, Gaken J, Devereux S, Kemeny M, Mufti G, Farzaneh F (2005) IL-2/B7.1 (CD80) fusagene transduction of AML blasts by a self-inactivating lentiviral vector stimulates T cell responses in vitro: a strategy to generate whole cell vaccines for AML. Mol Ther 11:120–131
56. Kaufman HL, Cohen S, Cheung K, DeRaffele G, Mitcham J, Moroziewicz D, Schlom J, Hesdorffer C (2006) Local delivery of vaccinia virus expressing multiple costimulatory molecules for the treatment of established tumors. Hum Gene Ther 17:239–244
57. Garnett CT, Greiner JW, Tsang KY, Kudo-Saito C, Grosenbach DW, Chakraborty M, Gulley JL, Arlen PM, Schlom J, Hodge JW (2006) TRICOM vector based cancer vaccines. Curr Pharm Des 12:351–361
58. Yu X, Fournier S, Allison JP, Sharpe AH, Hodes RJ (2000) The role of B7 costimulation in CD4/CD8 T cell homeostasis. J Immunol 164:3543–3553