Marking the 50th Anniversary of *Immunology*

Special regulatory T cell review: The resurgence of the concept of contrasuppression in immunoregulation

Thomas Lehner

Mucosal Immunology Unit, Kings College London at Guy's Hospital, Guy's Hospital, London, UK

doi:10.1111/j.1365-2567.2007.02780.x
Received 24 October 2007; accepted 1 November 2007.
Correspondence: Dr T. Lehner, Mucosal Immunology Unit, Kings College London at Guy's Hospital, Guy's Tower 28th Floor, Guy's Hospital, London SE1 9RT, UK. Email: thomas.lehner@kcl.ac.uk

Summary

The original concept of contrasuppression (CS) is evident in many immunoregulatory mechanisms. Inhibition of suppressor activity – CS – may be critical in microbial infection and autoimmunity. The major cellular interactions involved in suppression are the $CD25^+$ $FoxP3^+$ $CD4^+$ T regulatory cells, programmed death-1 (PD-1) : PD-L1/L2 and cytotoxic T lymphocyte antigen-4 (CTLA-4) : CD80/86 pathways. These cellular functions are affected by dendritic cells (DC) and a complex array of cytokines of which interleukin (IL)-2, IL-10, IL-6 and transforming growth factor-β (TGF-β) are especially significant. Inhibition of regulatory cells, suppressor pathways or cytokines, is consistent with CS and can be attributed to IL-6, IL-2, PD-1 or PD-L-1 antibodies, blockade of CTLA-4 : CD80/86 pathway, inhibition of CD40–CD40L pathways, and TGF-β, IL-10 antibodies. Contrasuppression may regulate innate immunity by Toll-like receptor expressed not only in non-cognate DC, monocytes, natural killer cells and γδ T cells but also in adaptive T cells. Furthermore, cross-talk between innate and adaptive immunity may be facilitated by contrasuppressor activity.

"What's in a name?
That which we call a rose by any other name would smell as sweet."
From *Romeo and Juliet* (II, 47–8) W. Shakespeare

Keywords: contrasuppression; immunoregulation; suppression

Background

The concept of contrasuppression (CS) was introduced by Richard Gershon and his colleagues a quarter century ago as a novel immunoregulatory activity which inhibits suppressor T-cell functions in mice.[1] The experimental approach taken was that the genetic program of immunologically competent cells express unique surface molecules that carry functional information.[2] The $Ly\text{-}1^+$ 2^{-*} phenotype was programmed to induce effector cells, which in turn induce antibodies, delayed hypersensitivity and cytotoxic functions.[2,3] Positive signals produced by most $Ly1^+$ cells, however also contained a small subset of $I\text{–}J^{+*}$ cells whose function was to activate suppressor cells, which counteracted the $Ly\text{-}1^+$ cells[4]. The $Ly1^-$ 2^{+*} cells, functioned predominantly to suppress immune responses, but a small subset of $Ly1^-2^+$ $I\text{–}J^+$ cells induced acceptor cells that were $Ly1^+$ 2^+ $I\text{–}J^+$. This phenotype distinguished it from T helper cells.

Both the inducer cell or its biologically active mediator and its acceptor cell were required for the expression of CS. As contrasuppressor cells could block the suppressive activity of cell-free mediators released by Ly2 suppressor T cells, the mechanism of CS was either separate from or in addition to the inactivation of suppressor cells themselves. The effect of suppressor cells on both helper and effector T cells was inhibited interfering with cell-mediated functions, such as delayed hypersensitivity.[5,6] CS have been studied in antibody production,[1,7,8] autoimmunity,[9] oral tolerance,[10] and tumour regression.[11] The cells secrete an antigen-specific CS factor which appeared to have comparable functions to those of the cells of

*Updated terminology $Ly1^+$ = $CD5^{high}$, $Ly1^-$ $CD5^{low}$; $Ly2^+$ = CD8α; I–J was defined by antibodies but the gene was not identified (Green DR, personal communication).

origin.[12] Surprisingly for its time, a murine T-cell hybridoma was generated, which constitutively released CS factor specific for the TNP hapten.[13] Both, suppressor cells and CS cells generated corresponding suppressor and CS-factors; the suppressor factors inhibited the CS effector T cells in an adoptive cell transfer assay, and the CS-factors protected the effector T cells from the suppressor T cells.[14] The lectin Vicia villosa played an important part in these experiments as the lectin binds CS cells and was used to separate these cells.[15]

The human counterpart of CS followed a series of cell depletion and reconstitution experiments involving $CD4^+$ and $CD8^+$ T-cell interactions.[16,17] As in the mouse experiments, human $CD8^+$ T cells were separated on VV-coated plates into VV adherent and non-adherent cells. Reconstitution of $CD4^+$ T cells with CD8 VV^+ cells and streptococcal antigen elicited significant helper factors, which was not evident when unfractionated $CD8^+$ or CD8 VV^- cells were used. Moreover, a dose-dependent contrasuppressor activity was demonstrated with $CD8^+$ VV^+ T cells in the presence of $CD8^+$ VV^- suppressor T cells, using radioassay for dinitrophenylated (DNP) antibodies when treated with DNP-streptococcal antigen in the presence of B cells.[16,18,19] The $CD8^+$ VV^+ cell function was most effective if the cells were added to the culture of B cells, $CD4^+$ T cells and monocytes before the $CD8^+$ VV^- cells. The target of $CD8^+$ VV^+ cells or their factors were the $CD4^+$ T cells. The potential role of CD8 VV^+ cells *in vivo* has been explored in patients with active systemic lupus erythematosus, as well as in the synovial fluid of patients with rheumatoid arthritis.[20,21] A significant increase in CD8 VV^+ cells was found in these patients consistent with CS playing a part in autoimmune diseases.

The concept of contrasuppression incorporated in immunoregulation

With the onset of cloning over 20 years ago $CD4^+$ helper and $CD8^+$ T-cell cytotoxic cells were readily cloned but not CD8 suppressor cells. As a consequence all work on suppressor cells and consequently contrasuppressors was discontinued. However, as genetic and phenotypic analysis of the control of immunity has advanced, the mechanism of helper and suppressor immune responses have made significant progress. Immunoregulation is critical in maintaining the balance between immunity and tolerance, which are involved not only in preventing infection, but also in limiting collateral immune-mediated tissue damage. Tolerance to the host tissues has to be safeguarded to prevent autoimmune reactions. The following short account will review the involvement of CS as an essential part of control and fine modulation of immunity and tolerance. Regulation of the inhibitory pathways will determine if immunogenicity will be suppressed, maintained or enhanced.

The re-discovery of suppressor cells – $CD25^+$ $FoxP3^+$ $CD4^+$ Treg cells

The resurgence of suppression as a regulatory mechanism in the control of immunity was the discovery of $CD25^+$ $CD4^+$ T regulatory (Treg) cells.[22] At least two types of regulatory cells have been identified. Natural $CD4^+$ Treg cells that express constitutively CD25, cytotoxic T lymphocyte antigen-4 (CTLA-4) and glucocorticoid inducible tumour necrosis factor (GITR). The transcription factor FoxP3 is required for generation of natural Treg cells and is the specific marker of this cell.[23] Natural Treg cells develop in the thymus and are found in the peripheral lymphoid tissue. The mechanism of suppression is mediated by interleukin (IL)-10, transforming growth factor-β (TGF-β) or CTLA-4 and has been extensively studied in mice and a variety of infections.[24] Inducible Treg cells develop from conventional $CD4^+$ T cells under the influence of cytokines or interference with costimulatory signals; these may result in TR1 or T helper 3 (Th3) regulatory cells. Indeed, high concentrations of TGF-β, IL-10 or IL-2 may induce $CD4^+$ CD25- T cells to develop suppressive function[25] and TGF-β induces FoxP3 transcription factor.[26]

Recently, one of the early workers reporting on suppression and CS demonstrated that apoptotic cells induce $CD8^+$ suppressor T cells without priming $CD4^+$ T cells for immunity.[27] The mechanism responsible for suppressing immunity was production of TRAIL (tumour necrosis factor related apoptosis-inducing ligand) by the suppressor $CD8^+$ T cells. Necrotic cells normally stimulate $CD4^+$ T-cell mediated immunity and do not induce TRAIL by $CD8^+$ T cells, unless antigen is presented in the absence of $CD4^+$ T cells.[27] They raised the possibility that $CD8^+$ T suppressor factor secreted by $CD8^+$ T suppressor cells[7,28,29] may be accounted for by TRAIL. The difficulties encountered in identifying the $CD8^+$ T suppressor cells may have been due to the regulatory activity of conventional $CD4^+$ helper and $CD8^+$ cytotoxic T cells. Thus, $CD8^+$ regulatory cell is a 'helpless' $CD8^+$ T cell.

Immunoregulation of suppression

The $CD25^+$ $FoxP3^+$ $CD4^+$ T regulatory cells prevent autoimmune responses, as well as over-reaction of the immune system.[22] The T regulatory cells suppressing immune responses, however in turn require control to enable immunity and tolerance to be modulated according to the needs of controlling infection and immunity (Table 1). Cytokines modulate most immune responses and IL-6[30] and IL-2[31] can reverse the suppressor activity of Treg cells resulting in CS. Targeting TGF-β or IL-10 by corresponding antibodies can also control immune responses in chronic infection.[24] Similarly, blockade of CTLA-4 enhances host immunity.[32,33] GITR ligand or

Table 1. Inhibition or modulation of regulatory cells, suppressor pathways and cytokines as a function of contrasuppression

Suppression	Contrasuppression
$CD25^+$ $FoxP3^+$ $CD4^+$ T cells	IL-6
	Inhibiting the CD40–CD40L pathway
	My D88-dependent signaling pathway of TLR2 in mice and TLR8 in humans
	TLR8 ligands-ssRNA and poly-G oligonucleotides
CTLA-4-CD80/CD86	Blockade by single-chain antibody to CTLA-4
PD-1:PD-L1/L2	IL-2
	PD-1 or PD-L1 antibodies
GITR	GITR ligands or antibodies
TGF-β, IL-10	TGF-β, IL-10 antibodies

antibodies engage GITR on effector T cells which mediates resistance to suppression by $CD4^+$ $CD25^+$ Treg cells.[34] An alternative mechanism has been postulated for the GITR–GITRL interaction, by boosting responder T cell function and enhancing resistance to Treg cell-mediated suppression.[35] This is probably the first time that the term 'CS' re-emerges in the title of a paper after 20 years of silence.

Immunoregulation of innate immunity may also involve inhibition of the $CD25^+$ $FoxP3^+$ $CD4^+$ T cells. IL-6 is an acute phase protein induced during inflammation and effectively inhibits generation of Treg cells.[30] This is an example of a cytokine produced by activating the innate immune system, suppressing Treg cells and eliciting TH17 cells generating IL-17. Thus, a contrasuppressor effect is combined with pro-inflammatory IL-17 production. Suppression by $CD4^+$ $CD25^+$ Treg cells of innate immune responses of natural killer (NK) cells, monocytes and neutrophils in *Helicobacter* infection of mice was dependent on the action of IL-10 and TGF-β.[36]

PD-1–PD-L1 inhibitory pathway in immunoregulation

Programmed death-1 (PD-1) is a transmembrane protein similar to CTLA-4, but does not bind to CD80 or CD86.[37] PD-1 is expressed by activated but not naive $CD4^+$ and $CD8^+$ T cells, and myeloid cells. PD-1 peaks 3 days after activation and then remains on the cell surface. The ligands to PD-1 are PD-L1 and PD-L2, which are members of the B7 family of receptors. Whereas PD-L1 is constitutively expressed on freshly isolated T and B cells, DC macrophages, and surprisingly on $CD4^+CD25^+$ Treg cells, PD-L2 expression is inducible only on DC and macrophages after cytokine stimulation.[38] PD-1–PD-L1 interaction inhibits $CD4^+$ T-cell proliferation and cytokine production.[39,40] Indeed, $PD\text{-}1^{-/-}$ mice develop autoimmune diseases, such as glomerulonephritis and lupus-like arthritis.[33,41]

Exogenous IL-2 or CD28 monoclonal antibodies (mAb) augmented IL-2 can overcome PD-L1-mediated inhibition.[42] Release from the inhibitory PD-1– PD-L1 pathway has been consistently achieved by antibodies to PD-1 or PD-L1 in humans and non-human primates infected by human immunodeficiency virus-1 (HIV-1), resulting in enhancement of T-cell immune responses to HIV-1.[43–45] A recent report suggests that blockade of the PD-1–PD-L1 pathway abrogates $CD4^+$ $CD25^+$ Treg cell activity, which is required for alloreactive suppression of $CD4^+$ $CD25^-$ T cells, both *in vitro* and *in vivo* for skin allograft rejection and graft versus host reaction.[46]

The role of Toll-like receptor (TLR) in immunoregulation

To counteract suppression by Treg cells during primary T-cell responses, MyD88-dependent signalling pathway of TLR is involved.[47] Inhibition of $CD25^+CD4^+$ Treg cells suppressive activity is mediated by TLR2 expressed by these cells in mice[48] and TLR8[49] in humans, enhancing $CD4^+$ T cell proliferation. The TLR2-dependent inhibition of Treg cells has been confirmed in $TLR2^{-/-}$ knock out mice. This finding has therapeutic implications as Pam3 Cys is a synthetic TLR2 ligand which can selectively inhibit Treg cell suppressor activity *in vivo*.[50] $CD4^+$ $CD25^+$ Treg cells in humans were also inhibited by TLR8 ligands, such as ssRNA and poly-G oligonucleotides and confirmed by siRNA knock down of TLR8 *in vitro* and inhibition of Treg cells *in vivo* anti-tumour immunity.[49] Immunoregulation is an essential feature in health and disease and the complexity of the mechanism of fine control between immunity and tolerance underlines the critical nature of this activity.

References

1 Gershon RK, Eardley DD, Derum S, Green DR, Shen FW, Yamauchi K, Cantor H, Murphy DB. Contrasuppression: a novel immunoregulatory activity. *J Exp Med* 1981; **153**:1533–46.

2 Cantor H, Boyse EA. Regulation of cellular and humoral immune responses by T-cell subclasses. *Cold Spring Harb Symp Quant Biol* 1977; **41**:23–32.

3 Cantor H, Gershon RK. Immunological circuits: cellular composition. *Fed Proc* 1979; **38**:2058–64.

4 Eardley DD, Murphy DB, Kemp JD, Shen FW, Cantor H, Gershon RK. Ly-1 inducer and Ly-1, 2 acceptor T cells in the feedback suppression circuit bear an I–J subregion controlled determinant. *Immunogenetics* 1980; **11**:549–57.

5 Green DR, Eardley DD, Kimura A, Murphy DB, Yamauchi K, Gershon RK. Immunoregulatory circuits which modulate responsiveness to suppressor signals: characterization of an effector cell in the contrasuppressor circuit. *Eur J Immunol* 1981; **11**:973–80.

6 Paliwal V, Freidman AM, Ptak W, Askenase PW. Monoclonal, antigen-specific, T cell contrasuppressor factor expresses determinants of TCR a-chain (not necessarily TCR β-chain), having a molecular mass of about 40 kDa. *J Immunol* 1994; **152**:2811–20.

7 Yamauchi KD, Murphy H, Cantor H, Gershon RK. Analysis of an antigen-specific H-2-restricted cell-free product(s) made by 'I–J–' Ly-2 cells (Ly-2 TsF) that suppresses Ly-2 cell-depleted spleen cell activity. *Eur J Immunol* 1981; **11**:913–8.

8 Braley-Mullen H. Characterization and activity of contrasuppressor T cells induced by type III pneumococcal polysaccharide. *J Immunol* 1986; **137**:2761–7.

9 Smith HRD, Green DR, Smathers PA, Gershon RK, Raveche ES, Steinberg AD. Induction of autoimmunity in normal mice by thymectomy and administration of polyclonal B cell activators: association with contrasuppressor function. *Clin Exp Immunol* 1983; **51**:579–86.

10 Suzuki I, Kiyono H, Kitamura K, Green DR, McGhee JR. Abrogation of oral tolerance by contrasuppressor T cells suggests the presence of regulatory T cell networks in the mucosal immune system. *Nature* 1986; **320**:451–4.

11 Flood PM, Freidman A, Freedman J, Horvat B, Reuter P, Ptak W. The role of contrasuppressor in tumor regression. *Immunol Res* 1988; **7**:12–22.

12 Ptak W, Bereta M, Marcinkiewicz J, Gershon RK, Green DR. Production of antigen-specific contrasuppressor cells and factor, and their use in augmentation of cell-mediated immunity. *J Immunol* 1984; **133**:623–8.

13 Friedman AM, Ptak E, Brewer E, Green DR, Reuter PA, Flood PM. Generation of a T cell hybridoma producing a contrasuppressor factor for contact sensitivity. *Immunology* 1990; **71**:282–9.

14 Ptak W, Bereta M, Ptak M, Gershon R, Green DR. Antigen-specific T contrasuppressor factor in cell-mediated immunity: interaction leading to eradication of the tolerant state. *J Immunol* 1984; **133**:1124–30.

15 Kimura A, Wigzell H, Holmquist G, Ersson B, Carsson P. Selective affinity fractionation of murine cytotoxic T lymphocytes (CTL). *J Exp Med* 1979; **149**:473–84.

16 Lehner T. The relationship between human helper and suppressor factors to a streptococcal protein antigen. *J Immunol* 1982; **129**:1936–40.

17 Lehner T, Lamb JR, Kontiainen S. Cell-bound helper and suppressor factors in primate lymphocytes. *Clin Exp Immunol* 1982; **47**:706–14.

18 Lehner T, Brines R, Jones T, Avery J. Detection of cross-reacting murine I–J like determinants on a human subset of $T8^+$ antigen binding, presenting and contrasuppressor cells. *Clin Exp Immunol* 1984; **58**:410–9.

19 Brines R, Lehner T. Antigen presentation by a subset of $T8^+$ Ia^+ cells to $T4^+$ helper cells. *Clin Exp Immunol* 1987; **67**:398–405.

20 Fortune F, Lehner T. Phenotypic expression of Vicia villosa binding T cell subsets, as markers of contrasuppressor cells in systemic lupus erythematosus. *Clin Exp Immunol* 1988; **74**:100–4.

21 Fortune F, Kingston J, Barnes C, Lehner T. Identification and characterisation of IgA and Vicia villosa-binding T cell subsets in rheumatoid arthritis. *Clin Exp Immunol* 1990; **79**:202–8.

22 Sakaguchi S. Naturally arising Foxp3-expressing $CD25^+$ $CD4^+$ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 2005; **6**:345–52.

23 Zheng Y, Rudensky AY. Foxp3 in control of the regulatory T cell lineage. *Nat Immunol* 2007; **8**:457–62.

24 Belkaid Y, Rouse BT. Natural regulatory T cells in infectious disease. *Nat Immunol* 2005; **6**:353–60.

25 Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. Natural and induced $CD4^+$ $CD25^+$ cells educate $CD4^+$ $CD25^-$ cells to develop suppressive activity: the role of IL-2, TGF-γ, and IL-10. *J Immunol* 2004; **172**:5213–21.

26 Chen W, Jen W, Hardegen N, Lei K, Marinos N, McGrady G, Wahl SM. Conversion of peripheral $CD4^+$ $CD25^-$ naive T cells to $CD4^+$ $CD25^+$ regulatory T cells by TGF-β induction of transcription factor FoxP3. *J Exp Med* 2003; **198**:1875–86.

27 Griffith TS, Kazama H, VanOosten RL, Earle JK, Herndon JM, Green DR, Ferguson TA. Apoptotic cells induce tolerance by generating helpless $CD8^+$ T cells that produce TRAIL. *J Immunol* 2007; **178**:2679–87.

28 Gershon RK, Kondo K. Infectious immunological tolerance. *Immunology* 1971; **21**:903–14.

29 Greene MI, Benacerraf B. Studies on hapten specific T cell immunity and suppression. *Immunol Rev* 1980; **50**:163–86.

30 Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006; **441**:235–8.

31 Baecher-Allan C, Viglietta V, Hafler DA. Human $CD4^+$ $CD25^+$ regulatory T cells. *Semin Immunol* 2004; **16**:89–97.

32 Griffin MD, Hong DK, Holman PO *et al.* Blockade of T cell activation using a surface-linked single-chain antibody to CTLA-4 (CD152). *J Immunol* 2000; **164**:4433–42.

33 Khoury SJ, Sayegh MH. The roles of the new negative T cell costimulatory pathways in regulating autoimmunity. *Immunity* 2004; **20**:529–38.

34 Stephens GL, McHugh S, Whitters MJ, Young DA, Luxenberg D, Carreno BM, Collins M, Shevach EM. Engagement of glucocorticoid-induced TNFR family-related receptor on effector T cells by its ligand mediates resistance to suppression by $CD4^+$ $CD25^+$ T cells. *J Immunol* 2004; **173**:5008–20.

35 Shevach EM, Stephens GL. The GITR–GITRL interaction: co-stimulation of contrasuppression of regulatory activity? *Nat Rev Immunol* 2006; **6**:613–8.

36 Maloy KJ, Salaun L, Cahill R, Dougan G, Saunders NJ, Powrie F. $CD4^+$ $CD25^+$ T_R cells cuppress innate immune pathology through cytokine-dependent mechanisms. *J Exp Med* 2003; **197**:111–9.

37 Agata Y, Kawaskai A, Nishimura H, Ishida Y, Tsubata T, Yagita H, Honjo T. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. *Int Immunol* 1996; **8**:765–72.

38 Yamazaki T, Akiba H, Koyanagi A, Azuma M, Yagita H, Okumura K. Blockade of B7-H1 on macrophages suppresses $CD4^+$ T cell proliferation by augmenting IFN-γ-induced nitric oxide production. *J Immunol* 2002; **175**:1586–92.

39 Freeman GJ, Wherry EF, Ahmed R, Sharpe AH. Reinvigorating exhausted HIV-specific T cells via PD-1-PD-1 ligand blocks. *J Exp Med* 2006; **203**:2223–7.

40 Latchman Y *et al.* PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol* 2001; **2**:261–8.

41 Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 1999; **11**:141–51.

42 Carter LL, Fouser LA, Jussif J *et al.* PD-1: PD-L inhibitory pathway affects both CD4+ and CD8+ T cells and is overcome by IL-2. *Eur J Immunol* 2002; **32**:634–43.
43 Trautmann L, Janbazian L, Chomont N *et al.* Upregulation of PD-1 expression on HIV-specific $CD8^+$ T cells leads to reversible immune dysfunction. *Nat Med* 2006; **12**:1198–202.
44 Petrovas C, Casazza JP, Brenchley JM *et al.* PD-1 is a regulator of virus-specific $CD8^+$ T cell survival in HIV infection. *J Exp Med* 2006; **203**:2281–92.
45 Day CL, Kaufmann DE, Kiepiela P *et al.* PD-1 expression on HIV-specific T cells associated with T-cell exhaustion and disease progression. *Nature* 2006; **443**:350–4.
46 Kitazawa Y, Fujino Q, Wang Q, Kimura H, Azuma M, Kubo M, Abe R, Li X-K. Involvement of the programmed death-1/programmed death-1 ligand pathway in $CD4^+CD25^+$ regulatory T-cell activity to suppress alloimmune response. *Transplantation* 2007; **83**:774–82.
47 Pasare C, Medzhitov R. Toll pathway-dependent blockade of $CD4^+CD25^+$ T cell-mediated suppression by dendritic cells. *Science* 2003; **299**:1033–6.
48 Liu H, Komai-Koma M, Xu D, Liew FY. Toll-like receptor 2 signalling modulates the functions of $CD4^+$ $CD25^+$ regulatory T cells. *Proc Natl Acad Sci U S A* 2006; **103**:7048–53.
49 Peng G, Guo Z, Kiniwa Y *et al.* Toll-like receptor 8-mediated reversal of $CD4^+$ regulatory T cell function. *Science* 2005; **309**:1380–4.
50 Sutmuller RPM, Morgan ME, Netea MG, Grauer O, Adema GJ. Toll-like receptors on regulatory T cells: expanding immune regulation. *Trends Immunol* 2006; **27**:387–93.