

# Big Mac Attack: Does It Play a Direct Role for Monocytes/Macrophages in Type 1 Diabetes?

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**T**ype 1 diabetes is conventionally thought to result from T-cell-mediated autoimmune destruction of pancreatic  $\beta$ -cells (1–3). Experimental and clinical evidence accumulated over the past two decades indicates that T-cells play a critical role in the pathogenesis of type 1 diabetes in both humans and the nonobese diabetic (NOD) mouse model of this disease (4). Indeed, the important contribution of T-cells toward the pathogenesis of type 1 diabetes has been supported not only by a variety of physiologic and histological studies but, in addition, through a number of immunotherapeutic-based studies involving selective targeting of T-cells (4,5). More recently, it has been shown that B-cells also play an important role in type 1 diabetes development (6), since NOD mice deficient in these cells do not develop insulinitis or overt diabetes (7), and that the depletion of B-cells with anti-CD20 antibody prevents disease (8).

Apart from diabetogenic T- and B-cells, emerging evidence suggests that macrophages are involved in the final stage of autoimmune-mediated  $\beta$ -cell destruction (9,10). For example, after monocyte depletion, passively transferred diabetogenic T-cells fail to induce diabetes, and activated macrophages can directly kill  $\beta$ -cells in vitro (10). However, direct evidence that activated monocytes/macrophages kill  $\beta$ -cells is minimal or lacking, depending on one's perspective. In the current issue of *Diabetes*, Martin et al. (11), using multiple transgenic mouse models, provide convincing evidence that monocytes can be recruited to pancreatic islets when the chemokine CCL2 is expressed transgenically in  $\beta$ -cells and that these immune system cells are capable of destroying  $\beta$ -cells, resulting in diabetes in the absence of mature T- and B-cells. Therefore, this observation adds monocytes/macrophages to the growing list of immune cells involved in islet cell destruction in type 1 diabetes.

There are two distinct phases for autoimmune-mediated diabetes: insulinitis and diabetes. In the early stages of insulinitis, mixed leukocytes including activated macrophages, B-cells, T-cells, and NK cells are attracted to the islets by chemokines. Chemokines are chemoattractant proteins (12,13) produced by cells in response to infection or cell damage (13). Leukocytes, such as lymphocytes, monocytes, and NK cells, expressing the appropriate receptors, migrate toward the source of chemokine produc-

tion. Chemokines are subdivided into four subfamilies (C, CC, CXC, and CX3C) based on the location of the first conserved  $\text{NH}_2$ -terminal cysteine residues (13). The largest group is the CC family, where two cysteine residues are immediately adjacent to each other.

CCL2, also known as monocyte chemoattractant protein-1 (MCP-1), is a CC chemokine produced by lymphocytes, monocytes, endothelial cells, and other cells in response to inflammatory stimuli (14). Through its receptor CCR2, MCP-1/CCL2 potently attracts monocytes, T-cells, and NK cells. Although MCP-1 is expressed in normal human and rodent islets (15), transgenic mice overexpressing MCP-1 under control of the insulin promoter develop an intense insulinitis, but overt diabetes was not seen in early studies (16). The temporal pattern of MCP-1 and other chemokine expression correlates with the progression of insulinitis and  $\beta$ -cell destruction in NOD mice (17).

Despite substantial indirect evidence that CCL2 is involved in the pathogenesis of type 1 diabetes, its precise role in the development of insulinitis and islet cell destruction is incompletely understood. Martin et al. (11) add to the accumulating evidence that CCL2 production and numbers of circulating monocytes play a more important role in the onset of diabetes than was previously suspected.

The investigators demonstrate that monocytes/macrophages are recruited in a concentration-dependent manner to the islets by transgenic expression of CCL2 in  $\beta$ -cells under the control of the insulin promoter. The CCL2-mediated monocyte recruitment requires the CCL2 receptor (CCR2), since deletion of CCR2 in RIPCC2 mice abolishes homing to the islets. Strikingly, and in contrast to previous studies (16,18), transgenic mice displaying high MCP-1 expression and serum MCP-1 levels not only develop insulinitis but also go on to islet cell destruction and clinical diabetes. The explanation for this apparent discrepancy may lie both in the relatively high level of MCP-1 expressed (up to 1.4 ng/ml in the serum) and in the fact that MCP-1 production in the Martin et al. (11) study was restricted to islets, whereas expression was more widespread in the study of Rutledge et al. (18). Importantly, to assess the influence of T- and B-cells, Martin et al. bred the RIPCC2 transgene onto a  $\text{Rag-1}^{-/-}$  background to generate mice expressing CCL2 in  $\beta$ -cells in the absence of mature T- and B-cells. Remarkably, the hybrid mice developed diabetes with a similar time course to that of immunocompetent  $\text{Rag1}^{+/+}$  controls.

Thus, although diabetogenic T- and B-cells may be central to the pathogenesis of type 1 diabetes under "physiological" conditions (2,19), the Martin et al. study extends data from several other laboratories (9,10,16,20), suggesting that monocytes/macrophages also may play a critical role. Most current therapies, such as the induction

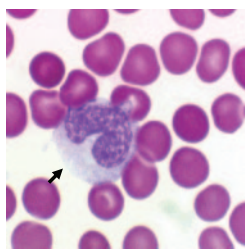
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See accompanying original article, p. 3025.



Circulating Monocyte

**FIG. 1. Arrow indicates a circulating monocyte on peripheral blood smear.**

of immunoregulatory T-cells, costimulatory blockade, and T- or B-cell depletion with anti-CD3 or -CD20 antibodies (5,8,19,21), target the lymphocyte arm of the autoimmune response to islets. One implication of the current study is that therapy directed at the antigen-presenting cells also may be effective. Indeed, a variety of MCP-1/CCL2 inhibitors are under development, and it has been shown that anti-CCL2 therapy can reverse other autoimmune disorders in animal models, such as murine demyelinating disease (22) and lupus nephritis (23). The Martin et al. study may provide a conceptual framework for the design of monocyte therapy aimed at interrupting insulinitis and the destruction of pancreatic  $\beta$ -cells. This study may also provide a rationale for monitoring CCL2 levels and circulating monocyte numbers in the blood as parameters to predict the disease onset and monitor the disease progression (Fig. 1).

However, caution should be exercised in evaluating and interpreting this study. Transgenic overexpression of CCL2 in  $\beta$ -cells does not necessarily reproduce the defect in human type 1 diabetes, although pancreatic islet tissue from patients with recent-onset type 1 diabetes exhibits macrophages and dendritic cells infiltrating the islets and the production of inflammatory cytokines (tumor necrosis factor- $\alpha$  and interleukin-1 $\beta$ ) (24). Moreover, in view of the redundancy of chemokine pathways, the efficacy of disrupting a single chemokine pathway remains to be determined. Nevertheless, further investigation of the potential for monocyte therapy of type 1 diabetes seems warranted.

## REFERENCES

1. Tisch R, McDevitt H: Insulin-dependent diabetes mellitus. *Cell* 85:291–297, 1996
2. Atkinson MA, Eisenbarth GS: Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet* 358:221–229, 2001
3. Bach JF: Autoimmunity and type I diabetes. *Trends Endocrinol Metab* 8:71–74, 1997
4. Roep BO: The role of T-cells in the pathogenesis of type 1 diabetes: from cause to cure. *Diabetologia* 46:305–321, 2003
5. Goudy KS, Tisch R: Immunotherapy for the prevention and treatment of type 1 diabetes. *Int Rev Immunol* 24:307–326, 2005
6. Silveira PA, Grey ST: B cells in the spotlight: innocent bystanders or major players in the pathogenesis of type 1 diabetes. *Trends Endocrinol Metab* 17:128–135, 2006
7. Serreze DV, Chapman HD, Varnum DS, Hanson MS, Reifsnyder PC, Richard SD, Fleming SA, Leiter EH, Shultz LD: B lymphocytes are essential for the initiation of T cell-mediated autoimmune diabetes: analysis of a new “speed congenic” stock of NOD.Ig mu null mice. *J Exp Med* 184:2049–2053, 1996
8. Xiu Y, Wong CP, Bouaziz JD, Hamaguchi Y, Wang Y, Pop SM, Tisch RM, Tedder TF: B lymphocyte depletion by CD20 monoclonal antibody prevents diabetes in nonobese diabetic mice despite isotype-specific differences in Fc $\gamma$  effector functions. *J Immunol* 180:2863–2875, 2008
9. Jun HS, Santamaria P, Lim HW, Zhang ML, Yoon JW: Absolute requirement of macrophages for the development and activation of  $\beta$ -cell cytotoxic CD8+ T-cells in T-cell receptor transgenic NOD mice. *Diabetes* 48:34–42, 1999
10. Calderon B, Suri A, Unanue ER: In CD4+ T-cell-induced diabetes, macrophages are the final effector cells that mediate islet beta-cell killing: studies from an acute model. *Am J Pathol* 169:2137–2147, 2006
11. Martin AP, Rankin S, Pitchford S, Charo IF, Furtado GC, Lira SA: Increased expression of CCL2 in insulin-producing cells of transgenic mice promotes mobilization of myeloid cells from the bone marrow, marked insulinitis, and diabetes. *Diabetes* 57:3025–3033, 2008
12. Baggiolini M, Loetscher P: Chemokines in inflammation and immunity. *Immunol Today* 21:418–420, 2000
13. Bendall L: Chemokines and their receptors in disease. *Histol Histopathol* 20:907–926, 2005
14. Oppenheim JJ, Zachariae CO, Mukaida N, Matsushima K: Properties of the novel proinflammatory supergene “intercrine” cytokine family. *Annu Rev Immunol* 9:617–648, 1991
15. Piemonti L, Leone BE, Nano R, Sacconi A, Monti P, Maffi P, Bianchi G, Sica A, Peri G, Melzi R, Aldrighetti L, Secchi A, Di CV, Allavena P, Bertuzzi F: Human pancreatic islets produce and secrete MCP-1/CCL2: relevance in human islet transplantation. *Diabetes* 51:55–65, 2002
16. Grewal IS, Rutledge BJ, Fiorillo JA, Gu L, Gladue RP, Flavell RA, Rollins BJ: Transgenic monocyte chemoattractant protein-1 (MCP-1) in pancreatic islets produces monocyte-rich insulinitis without diabetes: abrogation by a second transgene expressing systemic MCP-1. *J Immunol* 159:401–408, 1997
17. Ogliari AC, Caldara R, Socci C, Sordi V, Cagni N, Moretti MP, Dell’acqua A, Mercalli A, Scavini M, Secchi A, Bonifacio E, Bosi E, Piemonti L: High levels of donor CCL2/MCP-1 predict graft-related complications and poor graft survival after kidney-pancreas transplantation. *Am J Transplant* 8:1303–1311, 2008
18. Rutledge BJ, Rayburn H, Rosenberg R, North RJ, Gladue RP, Corless CL, Rollins BJ: High level monocyte chemoattractant protein-1 expression in transgenic mice increases their susceptibility to intracellular pathogens. *J Immunol* 155:4838–4843, 1995
19. Shoda LK, Young DL, Ramanujan S, Whiting CC, Atkinson MA, Bluestone JA, Eisenbarth GS, Mathis D, Rossini AA, Campbell SE, Kahn R, Kreuwel HT: A comprehensive review of interventions in the NOD mouse and implications for translation. *Immunity* 23:115–126, 2005
20. Hutchings P, Rosen H, O’Reilly L, Simpson E, Gordon S, Cooke A: Transfer of diabetes in mice prevented by blockade of adhesion-promoting receptor on macrophages. *Nature* 348:639–642, 1990
21. Staeva-Vieira T, Peakman M, von Herrath M: Translational mini-review series on type 1 diabetes: immune-based therapeutic approaches for type 1 diabetes. *Clin Exp Immunol* 148:17–31, 2007
22. Karpus WJ, Kennedy KJ, Fife BT, Bennett JL, Dal Canto MC, Kunkel SL, Lukacs NW: Anti-CCL2 treatment inhibits Theiler’s murine encephalomyelitis virus-induced demyelinating disease. *J Neurovirol* 12:251–261, 2006
23. Hasegawa H, Kohno M, Sasaki M, Inoue A, Ito MR, Terada M, Hieshima K, Maruyama H, Miyazaki J, Yoshie O, Nose M, Fujita S: Antagonist of monocyte chemoattractant protein 1 ameliorates the initiation and progression of lupus nephritis and renal vasculitis in MRL/lpr mice. *Arthritis Rheum* 48:2555–2566, 2003
24. Uno S, Imagawa A, Okita K, Sayama K, Moriwaki M, Iwahashi H, Yamagata K, Tamura S, Matsuzawa Y, Hanafusa T, Miyagawa J, Shimomura I: Macrophages and dendritic cells infiltrating islets with or without beta cells produce tumour necrosis factor-alpha in patients with recent-onset type 1 diabetes. *Diabetologia* 50:596–601, 2007