OBJECTIVE—The complement system contributes to autoimmune injury, but its involvement in promoting the development of autoimmune diabetes is unknown. In this study, our goal was to ascertain the role of complement C3 in autoimmune diabetes.

RESEARCH DESIGN AND METHODS—Susceptibility to diabetes development after multiple low-dose streptozotocin treatment in wild-type (WT) and C3-deficient mice was analyzed. Bone marrow chimera mice lacking C3 and expressing C3-deficient bone marrow cells showed that bone marrow cell–derived C3, and not serum C3, is involved in the induction of diabetes in this model.

RESULTS—Coincident with the induced elevations in blood glucose levels, we documented alternative pathway complement component gene expression within the islets of the diabetic WT mice. When we repeated the experiments with C3-deficient mice, we observed complete resistance to disease, as assessed by the absence of histologic insulitis and the absence of T-cell reactivity to islet antigens. Studies of WT chimera bearing C3-deficient bone marrow cells showed that bone marrow cell–derived C3, and not serum C3, is involved in the induction of diabetes in this model.

CONCLUSIONS—The data reveal a key role for immune cell–derived C3 in the pathogenesis of murine multiple low-dose streptozotocin-induced diabetes and support the concept that immune cell-mediated diabetes is in part complement-dependent.

Diabetes 59:2247–2252, 2010

Type 1 diabetes is a T-cell–dependent autoimmune disease in which islet antigens are presented by antigen-presenting cells (APCs) to autoreactive T cells, breaking self tolerance (1,2). After attraction to the pancreas, the autoreactive CD4 T cells cause β-cell injury in part through secreting proinflammatory cytokines that directly act on the islet cells (3), as well as by activating macrophages that amplify injury (4).

In previous work, we showed that during cognate T cell/APC interactions, immune cell–derived complement activates locally, yielding C3a and C5a that bind to C3a/C5a receptors (C3aR/C5aR) on both partners (5). The resultant G-protein–coupled receptor (GPCR) signaling further activates the APCs (upregulating costimulatory molecule expression and innate cytokine production) and directly induces survival and proliferation of the responding T cells. These concepts apply to in vivo immunity as T-cell responses to autoantigens (6–8), transplant antigens (9–12), and viruses (5,13) are diminished in mice in which immune cells are deficient in C3 or C3aR/C5aR, whereas T-cell immunity is enhanced in mice in which immune cells are deficient in the cell surface complement regulatory protein decay-accelerating factor (DAF, CD55) (8,10).

These results, along with a multitude of reports documenting that complement contributes to autoimmune injury (14–16), prompt the question of the possible involvement of the complement effectors in promoting the development of T-cell–mediated diabetes. This gap in the understanding of the function of complement in type 1 diabetes is unexpected, given that complement effectors, in particular C3a and C5a, are potent proinflammatory mediators and that inflammation has long been linked in the pathogenesis of type 1 diabetes.

To test the role of complement C3 on the development of T-cell–mediated diabetes, we employed an established model using multiple low-dose streptozotocin (MLDS) treatment. We chose the MLDS model over the NOD model because C3 and the diabetes susceptibility genes in the NOD strain are closely linked on chromosome 17 (17,18), thus impairing our ability to produce C3-deficient NOD animals. Streptozotocin (STZ), a toxin that binds to the GLUT2 receptor on pancreatic β-cells, has been used for decades to induce diabetes in rodent models (19). When administered at a single high dose (Hi-STZ, 180 mg/kg), it induces necrosis of the β-cells without leukocytic infiltrate. Collapsed islets and elevated serum glucose levels are detectable within 2–3 days (20). In contrast, when STZ is administered as multiple low doses (MLDS, 40 mg/kg daily for 5 days), it induces distortion of the islet architecture in conjunction with mononuclear cell infiltration. Although elevated serum glucose can be detected as early as day 7, typically 2 to 3 weeks are required for sustained diabetes (19). Rather than necrosis, apoptosis is the underlying mechanism of islet cell death, documented by findings that animals deficient in islet-associated caspase-3 are resistant to STZ effects (21). Current concepts are that apoptosis provides an environment in which islet autoantigens can be processed and presented by infiltrating APCs. Immune cell-mediated injury by autoreactive T cells that have escaped thymic deletion is the dominant pathogenic mechanism (22). Consistent with this hypothesis,
studies in the early 1980s demonstrated that T-cell–depleted or –deficient (nude) animals are resistant to MLDS-induced diabetes (23–25), and that T cells from animals with MLDS-induced disease can transfer diabetes to naive mice (26,27).

Herein we report that immune cell C3 is required for MLDS-induced diabetes, and strikingly, that the C3 must derive from immune cells rather than from the serum. Our results suggest that further studies are warranted in autoimmune diabetes in humans.

**RESEARCH DESIGN AND METHODS**

Reagents and antibodies. Anti-mouse CD45.1-PE, CD45.2-PerCP-Cy5.5, anti-mouse IFN-γ and biotinylated anti-IFN-γ mAb, anti-Annexin V-PE (BD Biosciences; San Jose, CA); anti-mouse C3-FITC (MP Biomedicals; Solon, OH); alkaline phosphatase-conjugated antibiotin antibody (Vector Laboratories; Burlingame, CA); streptavidin–HRP conjugate (Dako; Carpinetria, CA); collagenease P (Roche; Mannheim, Germany); zymosan A (Sigma Aldrich; St. Louis, MO); streptozotocin (Alexis Biochemicals; Farmingdale, NY).

**Mice.** BALB/c (H-2d), C57BL/6 (H-2b), B6.SJL-Pgmr–Pepc–Boyd (CD45.1), B6.C3–/–, and Rag-1–/– (B6.129S Rag-1<sup><sup>–/–</sup></sup>), male mice were purchased from Jackson Laboratory (Bar Harbor, ME). B6.C3–/– mice were backcrossed (>10 generations) to BALB/c to obtain BALB/c.C3–/–. C3 deficiency was confirmed via zymosan A C3-binding assay (11). Male mice were used at 6 to 10 wks of age, housed under specific-pathogen–free conditions, and treated in strict compliance with regulations established by the Institutional Animal Care and Use Committee.

**Diabetic model, islet isolation, and islet transplantation.** To induce diabetes, male mice (6–10 weeks of age) were injected intraperitoneally for 5 consecutive days with streptozotocin (10 mg/kg) dissolved in cold 0.1 mol/l citrate buffer pH 4.5 as previously described (26). Tail-vein glucose was measured between 10 A.M. and 12:00 P.M., and mice were considered diabetic if levels were >200 mg/dL in two consecutive measurements on the OneTouch Ultra Blood Glucose Meter (LifeScan; Milpitas, CA). In some experiments, mice were treated with a single 180-mg/kg body weight intraperitoneal injection of STZ. Islet isolation and transplantation were previously described (28). Isolated islets from male B6 mice were cultured overnight and incubated with STZ (0.5 mg/ml) for 1 h, washed, and transplanted beneath the renal capsule of diabetic B6 recipients (29). Islets were transplanted 10 days after initiating MLDS or 5 days after HI-STZ treatment in the recipient mouse. Intraportal glucose tolerance testing was performed on day 7 after transplantation and the area under the curve (AUC) was calculated.

**General of bone marrow chimera mice.** Bone marrow (BM) cells were collected from male WT or C3–/– mice of B6 background. Recipient male B6 mice had been lethally irradiated with 900 rad (2 doses of 450 rad with a 3-h resting period) from a cesium source using a Mark I Model 137Cs irradiator (11). Male mice were used at 6 to 10 wks of age, housed under specific-pathogen–free conditions, and treated in strict compliance with regulations established by the Institutional Animal Care and Use Committee.

**Adoptive cell transfer.** Splenocytes from male WT or C3–/– mice of B6 background were obtained by gently grinding moistened spleen through a 70-µm filter and washing the cells. Erythrocytes were lysed with ACK Lysis Buffer (Invitrogen; Carlsbad, CA). Splenocytes were resuspended in sterile PBS at a concentration of 3 × 10<sup>6</sup>/200 µl for intraperitoneal transfer into male B6.C3–/– mice. Twenty-four hours after adoptive transfer, recipient B6.C3–/– mice were treated with MLDS.

**Annexin V staining.** Isolated islets were cultured overnight with STZ (0.5 mg/ml) at 37°C in humidified air and 5% CO<sub>2</sub>. Islets were disrupted into a 70-µm-thick sections and islet morphology and leukocyte infiltration were assessed by H&E staining. Islets were graded by blinded investigators for severity of insulitis on a scale of 0–4: islets devoid of mononuclear cells = 0; minimum focal islet infiltrate = 1+; peri-islet infiltrate of <25% of islet circumference = 2+; minimum intrasial infiltrate and <50% of islet area = 3+; intrasial infiltrate >50% of islet area = 4+. The insulin score (%) for each group was calculated as sum of (1× number of islets with 1+; 2× number of islets with 2+; 3 × number of islets with 3+; 4 × number of islets with 4+) divided by 4 × total number of islets scored (30). The calculated ratio represents the insulin score percentage and was expressed as the mean ± SEM. Each study group included 3 mice with a minimum of 10 islets scored.

**Statistics.** Results are expressed as mean ± SEM, unless stated otherwise. Differences in gene expression were calculated using the nonparametric Mann-Whitney U tests. P < 0.05 was considered statistically significant. Statistical analysis was performed with the SPSS Version 16.0 software package (SPSS; Chicago, IL).

**RESULTS**

**MLDS induces T-cell–mediated autoimmune diabetes.** To verify that MLDS is T-cell dependent in our pathogen-free colony [contrasting with previous work done in the 1980s in which experiments were not done in a specific-pathogen–free environment (25,31)] we injected WT, RAG1<sup>–/–</sup>, and nude B6 mice with MLDS. In all WT mice, we detected progressively elevated serum glucose levels beginning on experimental day 7 and all became diabetic by experimental day 17. In contrast, we found that none of the RAG1<sup>–/–</sup> and none of the nude mice developed diabetes (Fig. 1A). In control experiments, we observed that Hi-STZ (which directly destroys islet tissue) induced diabetes by experimental day 7 comparably in WT and RAG1<sup>–/–</sup> mice (Fig. 1A). On H&E-stained pancreas tissues obtained on experimental day 19, we found significant intra- and peri-islet mononuclear infiltration in the pancreas of all WTs with a mean insulin score of 50.4 ± 6.5%, whereas we noted intact islets with no mononuclear cell infiltrates in all RAG1<sup>–/–</sup> mice (Fig. 1B).

To test whether the MLDS protocol induced islet-reactive T-cell autoimmunity, we reasoned that after syngeneic islet transplantation, the primed islet-reactive, cellular immune response would rapidly destroy the transplanted tissue and induce recurrent diabetes. To test this hypothesis, we isolated islets from WT B6 mice, pretreated them in vitro with STZ to facilitate neoantigen expression (29), and then transplanted 500 islets under the kidney capsules of syngeneic MLDS-induced diabetic B6 mice. We injected identically-treated islets into HI-STZ diabetic B6 recipient mice as controls. We observed that after transplantation, all animals rendered diabetic by either MLDS or Hi-STZ initially significantly lowered their serum glucose values by day 2 after transplantation, demonstrating that the transplanted islets were functional (Fig. 1C and D). Subsequently, the serum glucose of all of the transplanted MLDS-treated animals increased to pretransplant values within 1 week after transplantation (Fig. 1C). In contrast, in the HI-STZ-treated mice, we found that islet transplantation markedly reduced and stabilized lower serum glucose in all animals and fully normalized serum glucose in 6 of 8 mice (Fig. 1D). On day 7 after transplantation, the MLDS recipients had a significantly impaired insulin response after intraperitoneal glucose load compared with the HI-STZ–treated recipients (Fig. 1E). When we examined the...
pancreas tissue, we found mononuclear cell infiltration in the transplanted islets from the MLDS-treated mice, but not in the Hi-STZ–treated animals (Fig. 1F). Control experiments with islets that were not treated in vitro with STZ prior to transplantation into MLDS mice resulted in prolonged euglycemia (n = 3; data not shown). Together these experiments support the concept that MLDS induces autoimmune diabetes, whereas Hi-STZ induces diabetes through an islet-toxic mechanism.

C3 is required for MLDS-induced diabetes. Because we (8,32,33) and others (34–36) have shown that immune cell-produced complement exerts control over T-cell autoimmunity in other models, we assessed the kinetics of complement component gene expression in total pancreatic tissue after MLDS administration using qRT-PCR. We detected gene transcripts for complement components C3 and factor B in pancreatic tissue obtained on experimental day 5 of MLDS. We noted that other complement components, receptors, and regulators, including factor D, C3aR1, C5aR1, and decay-accelerating factor (DAF) were detectable, but were not increased during the same time period (Fig. 2). We did not detect C3 or factor B transcripts from purified islets obtained from untreated animals or from mice treated with MLDS on days 5 or 10 (data not shown), suggesting that the pancreatic C3–derived from peri-islet immune cell infiltrates rather than from islet cells.

To test whether C3 is required to induce diabetes after MLDS, we administered MLDS to C3−/− mice on both C57BL/6 (n = 12) and BALB/c (n = 7) background. In contrast to the WTs (C57BL/6 n = 11, BALB/c n = 11) in which blood sugars exceeded 200 mg/dl at experimental day 11 in all mice, none of the C3−/− mice from either background developed hyperglycemia (Fig. 3A). Although we found histologic evidence of insulitis on experimental day 19 after MLDS in WT mice, we did not observe mononuclear infiltration within the islets of C3−/− animals (Fig. 3B). The mean insulin score was 50.4 ± 6.5% in WT versus 5.3 ± 2.7% in C3−/− mice (P < 0.01) (Fig. 3B). In control experiments to test whether islets lacking C3 limits susceptibility to the effects of STZ, we cocultured WT and C3−/− islets overnight with 0.5 mg/ml STZ. When we then tested the cultured islets for apoptosis, we found similar levels of Annexin V staining in WT islets and C3−/− islets, 50.1% vs. 56.3%, respectively (Fig. S1, available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db10-0044/DC1). In confirmation of equivalent susceptibility to STZ, all Hi-STZ treated C3−/− mice (n = 4) and WT mice (n = 7) developed diabetes with identical kinetics to the WT controls (Fig. 3A).

Because mononuclear cell chemoattractants CCL2 and CCL3, in addition to T-cell–derived IFN-γ and the innate cytokine IL-6, are implicated as mediators of insulitis, we measured their gene transcripts in pancreatic tissue of MLDS-treated WT and C3−/− mice. Compared with WT
C3 deficiency protects from MLDS-induced diabetes. A: Cumulative diabetes incidence and average blood glucose levels of WT or C3−/− mice on both the B6 and Balb/c background after administration of MLDS (n = 7–11 per group) or Hi-STZ (n = 3–4); **p < 0.01 (comparing blood glucose level of WT versus C3−/− on day 17). B: Histopathology of pancreata on experimental day 19 of MLDS showing mononuclear cell infiltration in WT, but not in C3−/− mice. Arrowheads indicate areas of infiltration; original magnification ×200. Mean insulitis scores (% ± SEM) of mice on experimental day 19 were obtained from 3 mice for each group. P < 0.01 (C) qRT-PCR of CCL2, CCL3, IFN-γ, IL-6, and macrophages (CD68) from total pancreata (normalized to cyclophilin) on experimental days 0, 5, and 10 after initiation of MLDS (n = 6 per group and time point) with C3−/− mice with lower expression compared with WT mice. Box and whisker plots show the medians and percentile values (10, 25, 75, and 90) for normalized mRNA.

Diminished T-cell immunity to islet antigens in C3−/− mice. To test the effect of C3 deficiency on the islet-reactive T-cell response, we cultured spleen cells from MLDS-treated WT and C3−/− mice (and from untreated controls) in the presence or absence of purified islet cells and measured cytokine production in culture supernatants 24 h later (Fig. 4). These analyses revealed islet-induced production of TNFα (experimental day 10) and IFN-γ (experimental day 19) in MLDS-treated WT mice. Splenocytes obtained from untreated mice did not respond to islet stimulation, confirming that the noted cytokine production was induced by MLDS. In contrast to the results in WT mice, we did not detect cytokines in cultures of day-19 spleenocytes obtained from MLDS-treated C3−/− mice. Other cytokines including IL-17 and IL-10 were barely detectable and not different between groups (data not shown).

MLDS-induced diabetes requires bone marrow cell-derived C3. Because immune cell–derived complement plays an integral role in adaptive T responses (13,37), we transplanted C3−/− (CD45.2) BM into lethally irradiated CD45.1 WT B6 mice (C3−/− BM→WT) to produce animals that contained serum C3, but possessed C3−/− BM cells. Conversely, we transplanted WT B6 CD45.1 BM into lethally irradiated CD45.2 C3−/− mice (WT BM→C3−/−) to produce animals deficient in serum C3, but with C3+ BM–derived cells. WT BM→WT chimeras on the B6 background were produced as controls. Staining peripheral blood for CD45.1/CD45.2 and analyzing the cells by flow cytometry (Fig. 5A) verified that the immune cells were >90% donor BM-derived. Zymosan C3 uptake assays (Fig. 5B) validated the presence or absence of C3 in the serum of each animal. We did not detect C3 in the sera of any WT BM→C3−/− chimeras (same as C3−/− controls), but we observed that all sera from C3−/− BM→WT and WT BM→WT chimeras was C3+ comparable to WT controls (Fig. 5B). When we administered MLDS to the chimeric animals, we found that only 2 of 8 C3−/− BM→WT chimeras developed diabetes, whereas all of the WT BM→C3−/− chimeras and all of the control WT BM→WT chimeras developed sustained hyperglycemia (Fig. 5C).

In separate “add back” experiments to test the requirement for spleen cell–derived C3 in MLDS-induced diabetes, we adoptively transferred 3 × 106 WT or control C3−/− spleen cells into C3−/− mice, and then treated all animals with MLDS. Although 2 of 3 C3−/− mice that received WT spleen cells became diabetic, none of 4 animals that received C3−/− spleen cells developed hyperglycemia (Fig. S2 in the online appendix).

DISCUSSION

Taken together, our findings indicate that immune cell–derived C3 is required for the development of diabetes in the MLDS model. We showed that in WT animals, MLDS-induced hyperglycemia and islet inflammation are associated with complement gene upregulation (Fig. 2). We then documented that C3-deficient mice from two different genetic backgrounds are resistant to MLDS-induced diabetes (Fig. 3). This protective phenotype occurs in the absence of islet inflammation (Fig. 3) and in association with diminished islet antigen-induced spleen cell–derived IFN-γ and TNFα production (Fig. 4). Using a bone marrow chimera strategy (and confirmed by spleen cell adoptive
antigen processing and presentation of autoantigens may contribute to the protective phenotype of the C3-deficient mice (40). Macrophage-derived C3, a key regulator of macrophage activation (41), may also be important in facilitating macrophage-mediated islet injury (4,15). These, among other effects of C3 on regulating innate and adaptive immunity, require additional study.

It is notable that NOD mice develop spontaneous autoimmune diabetes despite being C5 deficient (42), indicating that C5, C5a, and the membrane attack complex (43) are not required in the pathogenesis of diabetes in that model system. Whether C3 and/or its activation cleavage products, C3a, C3b, or C3dg are involved in the pathogenesis of diabetes in NOD mice is an issue that remains to be tested. However, the generation of C3−/− NOD mice is improbable because both C3 and the diabetes-susceptible H-2Kd genes are located on chromosome 17 (17,18).

Our data support the interpretation that MLDS-induced diabetes is, in part, an autoreactive, T-cell-mediated process. We showed that both T-cell-deficient nude mice and T- and B-cell-deficient Rag1−/− mice do not develop hyperglycemia despite administration of MLDS that is pathogenic in WT animals (Fig. 1). Our documentation of recurrent hyperglycemia after syngeneic islet transplantation of MLDS-induced diabetic animals, but not diabetic mice induced by HI-STZ (Fig. 1), supports this interpretation.

In summary, this work demonstrates an unanticipated key role for immune cell-derived C3 in the pathogenesis of murine autoimmune diabetes. These results argue that studies testing the function of immune cell-derived complement in human diabetes are warranted.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institutes of Health (AI-071185) and Juvenile Diabetes Research Foundation grant 5-2008-954 awarded to P.S.H.; KO8 AI 071038 to B.S.; NIH EY105476 and EY11288 to M.E.M.

No potential conflicts of interest relevant to this article were reported.

M.L. researched data and wrote the manuscript. N.Y. and S.S. researched data. B.M. and M.E.M. reviewed/editied the manuscript. P.S.H. and B.S. contributed to discussion and reviewed/editied the manuscript.

The authors thank Dr. Jonathan Bromberg, Mount Sinai School of Medicine, for critical reading of this manuscript.

REFERENCES

1. Shizuru JA, Taylor-Edwards C, Banks BA, Gregory AK, Fathman CG. Immunotherapy of the nonobese diabetic mouse: treatment with an antibody to T-helper lymphocytes. Science 1988;240:659–662
2. Christianson SW, Shultz LD, Leiter EH. Adoptive transfer of diabetes into immunodeficient NOD-scid/scid mice. Relative contributions of CD4+ and CD8+ T-cells from diabetic versus prediabetic NOD/Non-Thy-1a donors. Diabetes 1993;42:44–55
3. Rashinovitch A, Suarez-Pinzon WL. Cytokines and their roles in pancreatic islet beta-cell destruction and insulin-dependent diabetes mellitus. Biochem Pharmacol 1998;55:139–149
4. Martin AP, Rankin S, Pitchford S, Charo IF, Furtado GC, Lima 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 2008;57:3025–3033
5. Stranic MG, Liu J, Huang D, An F, Lalli PN, Maojun N, Shapiro VS, Dabyak GR, Heeger PS, Medof ME. Locally produced complement fragments C5a and C3a provide both proinflammatory and survival signals to naive CD4+ T cells. Immunity 2008;28:425–435
6. Yalcindag A, He R, Lauonnui D, Alenius H, Carroll M, Oettgen HC, Gesa BS. The complement component C3 plays a critical role in both Th1 and Th2 responses to antigen. J Allergy Clin Immunol 2006;117:1455–1461
7. Monach PA, Verschoor A, Jacobs JP, Carroll MC, Wagers AJ, Benoist C, Mathis D. Circulating C3 is necessary and sufficient for induction of autoantibody-mediated arthritis in a mouse model. Arthritis Rheum 2007; 56:2968–2974

8. Liu J, Lin F, Strainic MG, An F, Miller RH, Altuntas CZ, Heeger PS, Tuohy VK, Medof ME. IFN-gamma and IL-17 production in experimental autoimmune encephalomyelitis depends on local APC-T cell complement production. J Immunol 2008;180:5882–5889

9. Lalli PN, Zhou W, Sacks S, Medof ME, Heeger PS. Locally produced and activated complement as a mediator of alloreactive T cells. Front Biosci (Schol Ed) 2009;1:117–124

10. Pavlov V, Baelder H, Yuan S, Leisman S, Kwan WH, Lalli PN, Medof ME. Donor deficiency of decay-accelerating factor accelerates murine T cell-mediated cardiac allograft rejection. J Immunol 2008;181:4580–4589

11. Lalli PN, Strainic MG, Yang L, Lin F, Medof ME, Heeger PS. Locally produced C5a binds to T cell-expressed C5AR to enhance effector T cell expansion by limiting antigen-induced apoptosis. Blood 2008;112:1759–1766

12. Heeger PS, Lalli PN, Lin F, Yahijskikh A, Liu J, Muqim N, Xu Y, Medof ME. Decay-accelerating factor modulates induction of T cell immunity. J Exp Med 2005;201:1523–1530

13. Verschoor A, Brockman MA, Gadjeva M, Knipe DM, Carroll MC. Myeloid C3 determines induction of humoral responses to peripheral herpes simplex virus infection. J Immunol 2005;175:5363–5371

14. Holers VM. The spectrum of complement alternative pathway-mediated diseases. Immunol Rev 2008;223:300–316

15. 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 1990;348:639–642

16. Noorchashm H, Moore DJ, Lieu YK, Noorchashm N, Schlachterman A, Volanakis JE, Wetsel RA, Colten HR. Genetic disruption of the murine alternative complement pathway regulatory protein properdin abrogates diabetes mellitus in NOD mice. J Immunol 1999;163:75–79

17. Verschoor A, Brockman MA, Gadjeva M, Knipe DM, Carroll MC. Myeloid C3 determines induction of humoral responses to peripheral herpes simplex virus infection. J Immunol 2005;175:5363–5371

18. Leiter EH, Lee CH. Mouse models and the genetics of diabetes: is there complement at play? Diabetes 1998;47:50–56

19. Wang Z, Gleichmann H. GLUT2 in pancreatic islets: crucial target molecule in diabetes mellitus. Mol Cell Biol 2005;25:3620–3629

20. Kuhn KA, Cozine CL, Tomoska B, Robinson WH, Holers VM, Medof ME. Decay accelerating factor deficiency ameliorates collagen-induced arthritis in mice with experimental arthritis. J Immunol 2009;182:3141–3150

21. Kaya Z, Afanasyeva M, Wang Y, Dohnen KM, Schlitching J, Tretter T, Fairweather D, Holers VM, Rose NR. Contribution of the innate immune system to autoimmune myocarditis: a role for complement. Nat Immunol 2001;2:739–745

22. Kemper C, Atkinson JP, T cell-regulation with complements from innate and adaptive immune cells. Front Biosci 2001;6:739–745

23. Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Bullard EC. Multiple roles of complement in immunity and inflammation. Annu Rev Immunol 2000;18:461–495

24. Premanandan C, Storozuk CA, Clay CD, Lai L, Schlesinger LS, Phipps AJ. Complement protein C3 binding to Bacillus anthracis spores enhances phagocytosis by human macrophages. Microb Pathog 2006;40:306–314

25. Wang Z, Gleichmann H. GLUT2 in pancreatic islets: crucial target molecule in diabetes mellitus. Mol Cell Biol 2005;25:3620–3629

26. Eliss D, Frigozin H, Polak N, Rapoport M, Lohse AW, Cohen IR. Autoimmune diabetes induced by the beta-cell toxin STZ. Immunity to the 60-kDa heat shock protein and to insulin. Diabetes 1994;43:992–998

27. Kuhn KA, Cozine CL, Tomoska B, Robinson WH, Holers VM. Contribution of complement receptor CR2 deficiency to autoimmune myocarditis. J Immunol 2001;166:4381–4385

28. Premanandan C, Storozuk CA, Clay CD, Lai L, Schlesinger LS, Phipps AJ. Complement protein C3 binding to Bacillus anthracis spores enhances phagocytosis by human macrophages. Microb Pathog 2006;40:306–314

29. Walsworth DA, Roche WR, Lacy PE. Low-dose streptozocin-induced diabetes in mice. Acta Pathol Microbiol Scand [C] 1978;86C:277–282

30. Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Bullard EC. Multiple roles of complement in immunity and inflammation. Annu Rev Immunol 2000;18:461–495

31. Sandler D, Andersson A. Survival of intrasplenically implanted islets in mice with experimental insulitis and hyperglycemia. Diabetes 1982;31 Suppl. 4:78–83

32. Lalli PN, Strainic MG, Yang L, Lin F, Medof ME, Heeger PS. Decay-accelerating factor deficiency increases susceptibility to dextran sulfate sodium-induced colitis: role for complement in inflammatory bowel disease. J Immunol 2004;172:3836–3841

33. Hietala MA, Johnson IM, Tarkowski A, Kleinau S, Pako A, Medof M. Decay acceleration ameliorates collagen-induced arthritis in mice. J Immunol 2002;169:454–459

34. Kemper C, Atkinson JP, T cell-regulation with complements from innate and adaptive immune cells. Front Biosci 2001;6:739–745

35. Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Bullard EC. Multiple roles of complement in immunity and inflammation. Annu Rev Immunol 2000;18:461–495

36. Kuhn KA, Cozine CL, Tomoska B, Robinson WH, Holers VM. Contribution of complement receptor CR2 deficiency to autoimmune myocarditis. J Immunol 2001;166:4381–4385

37. Li K, Anderson KJ, Peng Q, Noble A, Lu B, Kelly AP, Wang N, Sacks SH, Zhou W. Cyclic AMP plays a critical role in C3a-receptor-mediated regulation of dendritic cells in antigen uptake and T-cell stimulation. Blood 2008;112:5964–5974

38. Zhou W, Patel H, Li K, Peng Q, Villiers MB, Sacks SH. Macrophages from C3-deficient mice have impaired potential to stimulate alloreactive T cells. Blood 2006;107:2461–2469

39. Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Bullard EC. Multiple roles of complement in immunity and inflammation. Annu Rev Immunol 2000;18:461–495

40. Premanandan C, Storozuk CA, Clay CD, Lai L, Schlesinger LS, Phipps AJ. Complement protein C3 binding to Bacillus anthracis spores enhances phagocytosis by human macrophages. Microb Pathog 2006;40:306–314

41. Walsworth DA, Roche WR, Lacy PE. Low-dose streptozocin-induced diabetes in mice. Acta Pathol Microbiol Scand [C] 1978;86C:277–282

42. Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Bullard EC. Multiple roles of complement in immunity and inflammation. Annu Rev Immunol 2000;18:461–495

43. Baxter AG, Cooke A. Complement lytic activity has no role in the pathogenesis of autoimmune processes. Proc Natl Acad Sci U S A 1980;77:6129–6133

44. Buschard K, Rygaard J. T-lymphocytes transfer streptozocin-induced diabetes mellitus in mice. Acta Pathol Microbiol Scand [C] 1978;86C:277–282