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Brief communication

The role of tissue adaptation and graft size in immune tolerance

Ehud Hauben [a], Maria Grazia Roncarolo [a,b], Elena Draghici [a], Uri Nevo [c,*]

[a] San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Italy
[b] Vita-Salute San Raffaele University, Via Olgettina 58, Milan 20132, Italy
[c] Section on Tissue Biophysics and Biomimetics, Laboratory of Integrative and Medical Biophysics, National Institute of Child Health and Human Development, National Institutes of Health, 13 South Drive Bethesda, MD 20892, USA

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Abstract

Understanding how immune tolerance is induced and maintained is critical for our approach to immune-related diseases. Ecoimmunity is a new theory that views the immune system–tissue interaction as a co-adapting predator–prey system. Ecoimmunity suggests that tissues adapt to the selective immune pressure during ontogeny and throughout life. Therefore, immune tolerance towards 'self' represents a symmetric balance between the propensity of the immune system to prey on 'self' cells, and the tissue's specific capacity to undergo phenotypic adaptations in order to avoid destructive immune interaction. According to this theory, we hypothesized that tissues of adult immune-deficient mice, which are not exposed to selective immune pressure, will not withstand immune activity and will therefore display higher susceptibility to graft rejection. To test this prediction, C57Bl/6 wild type female mice were rendered diabetic by streptozotocin and transplanted with syngeneic pancreatic islets isolated from either immune-deficient C57Bl/6 SCID or wild type females. Remarkably, recipients of islet grafts from immune-deficient syngeneic donors displayed significantly impaired glucose homeostasis compared to mice transplanted with islets of wild type donors ($p<0.001$, two way repeated measures ANOVA). The severity of this impairment was correlated with islet graft size, suggesting a capacity of transplanted islets to gradually acquire a tolerogenic phenotype. These findings support the view of graft survival that is based on 'natural selection' of tissue cells. In addition, we describe a new experimental system for molecular characterization of self-tolerance.

Keywords: Ecoimmunity; Immune-deficient mice; Islet transplantation; Diabetes; Immune tolerance; Graft size; Tissue adaptation

1. Introduction

Immune tolerance towards the host's own tissues is a basic characteristic of the immune system and is a prerequisite for its safe operation. In the last two decades, detailed knowledge of the intercellular and molecular mechanisms directing the induction and maintenance of immune tolerance [1] has been acquired. Nevertheless, a comprehensive model detailing our understanding of these mechanisms remains to be described [2]. The quest for a thorough model of self-tolerance is important not only for a desired theoretical scientific coherency, but also since better understanding of a 'healthy' immune system–tissue interaction may reflect on possible therapies for several diseases including rejection of transplants, degenerative and autoimmune diseases, and cancer [3].

We have recently suggested an alternative approach to the question of how immune tolerance is maintained, by outlining a new model termed 'Ecoimmunity' [4,5]. Ecoimmunity suggests that the immune system is not the only organ that continuously adapts to its environment. Rather, to survive under the selective pressure applied by a functional immune system, every cell must acquire properties that allow it to continuously avoid, modulate or specifically suppress autoimmune responses. Moreover, the interaction between immune cells and tissue cells is modeled as a co-evolving predator–prey system, identical to the interface of the immune system with pathogens [6]. Early in life, immune pressure dictates natural selection of cells that are capable of avoiding a destructive interaction with the immune system. The adaptation of tissue is assumed to occur by plasticity of phenotype. Tissue cells with an ability to adopt an advantageous phenotype survive, differentiate, and pass their phenotype on to daughter cells or induce it in neighboring cells. Cells that lack this ability are eliminated by a physiological autoimmune response [7,8].

* Corresponding author.
E-mail address: nevouri@mail.nih.gov (U. Nevo).

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As in macroscopic ecological systems, symmetric co-evolution throughout development is the most efficient mechanism to achieve a steady state condition between the immune system and the tissue. However, throughout life, autoimmune responses towards modified tissue cells (e.g., in the context of viral infections, traumatic injuries or degenerative diseases), although associated with a cost (immune-mediated tissue loss), result in elimination of aberrant or necrotic cells, and in adaptation and reacquired fitness of surviving tissue cells [7,9]. Ecoimmunity suggests that genetic background does not define 'self' or 'non-self' deterministically but rather that it defines the repertoire of possible expressed phenotypes of tissue and immune cells. These phenotypes are set, maintained, or altered according to a specific dialogue that is set within each individual. Different individuals therefore express different immune system–tissue dialogues, and these differences prevent the transfer of organs between individuals.

It is well established that the achievement of long-term graft acceptance (e.g., liver, skin, pancreatic islets) and the induction of transplantation tolerance is correlated to the size of the graft [10,11]. Accordingly, it has been recently demonstrated by Yasunami et al., that transplantation of 400, but not 200 syngeneic islets into the liver, was sufficient to establish normoglycemia in streptozotocin-injected diabetic mice. Moreover, intact islets with well-granulated β cells were histologically seen in mice receiving 400 islets, whereas damaged islets with degranulated β cells were observed in mice receiving 200 islets [12]. These data confirm that an immune response is mounted towards transplanted cells, even in the case of syngeneic islet transplantation. Large syngeneic grafts have the capacity to adapt to their new environment and accomplish the induction of immune tolerance, whereas smaller grafts fail to adapt and are therefore rejected.

Immune-deficient mice display various developmental and functional impairments, including diminished functional recovery from CNS injury [13,14], cognitive dysfunctions [15], and impaired neurogenesis and spatial learning abilities [16,17]. Ecoimmunity predicts that various organs and tissues in immune-deficient animals will display physiological dysfunctions due to the absence of immune mediated natural selection of competent cells. Accordingly, we predicted that the phenotype acquired by tissues of mice with normal immune functions renders them resistant to immune mediated destruction by the corresponding immune system. On the other hand, cells of immune-deficient mice do not necessarily adopt such a phenotype. We therefore suggest that syngeneic organ transplantation from immune-deficient donors into immune competent hosts will result in impaired graft survival. Here we tested this prediction *in vivo* in a murine model of pancreatic islets transplantation.

2. Methods

2.1. Animals

Female B6 SCID (B6.CB17-Prkdc^{scid}/SzJ) and wild type mice of the same genetic background were purchased from Charles River (Calco, Italy). All mice were kept under specific pathogen-free conditions. Glucose levels in the tail venous blood were quantified using the Glucometer Elite system (Bayer, Wuppertal, Germany) and were always measured in the morning. Diabetes was induced in B6 wild type mice by intravenous injection of streptozotocin (Sigma, St. Louis, MO) at 170 mg/kg. A diagnosis of diabetes was made after two sequential glucose measurements >350 mg/dl. All animal care procedures were performed according to protocols approved by the Hospital San Raffaele Institutional Animal Care and Use Committee (IACUC #255).

2.2. Syngeneic islet transplantation

After being cultured overnight at 37 °C, handpicked pancreatic islets (from SCID or wild type B6 mice) were transplanted (50, 100 or 200 islets/mouse) under the kidney capsule of recipient diabetic wild type mice, as previously described [18]. Blood glucose levels were monitored at indicated time points thereafter.

2.3. Glucose tolerance test (IVGTT)

60 days post-islet transplantation, following an overnight food deprivation, mice were injected intravenously with glucose solution (2 g/kg). Glucose blood levels were measured at indicated time points.

2.4. Statistical analysis

Differences between groups were assessed using repeated measures ANOVA and 2-tailed *t*-test.

3. Results

3.1. Transplantation of large syngeneic graft from immune-deficient mice results in graft acceptance with impaired glucose tolerance capacity

Mice in both groups received a transplant of 200 islets, regained normal blood glucose levels shortly after transplantation and remained

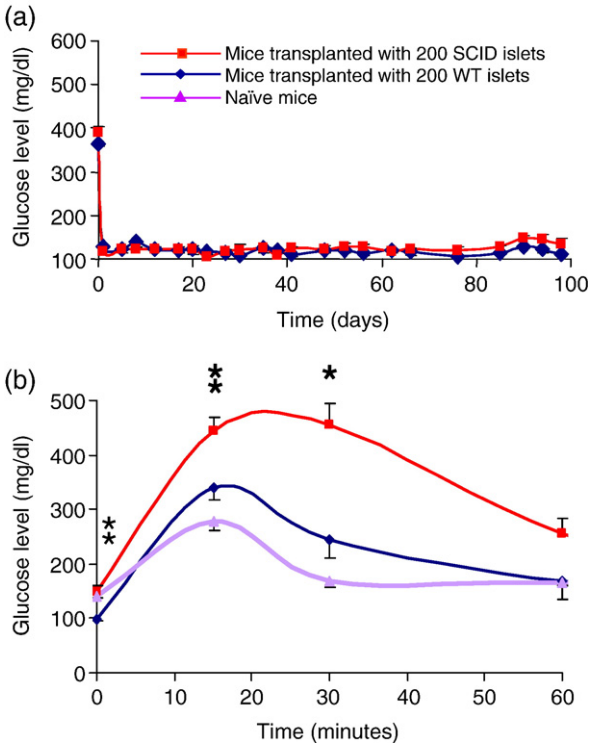

Fig. 1. Transplantation of 200 syngeneic islets from immune-deficient mice results in graft acceptance with impaired glucose tolerance capacity. WT mice were transplanted with 200 syngeneic islets isolated from either WT ($n=5$) or SCID mice ($n=5$). (a) Glucose levels were determined at indicated time points. (b) Intravenous glucose tolerance test was performed 60 days post transplantation (*$p<0.05$, **$p<0.01$; two-tailed *t*-test).

normoglycemic at least up to 4 months post transplantation (Fig. 1a). However, interestingly, while glucose tolerance capacity in mice receiving transplants of 200 WT islets was similar to that of control naive mice, mice receiving transplants of 200 SCID islets displayed significantly impaired glucose tolerance capacity (Fig. 1b; $p<0.001$ ANOVA). These results suggest that although syngeneic islets from SCID mice could survive and fulfill their function in WT recipients, due to a longer adaptation period to the immune competent environment, their functional capacity, possibly due to a lower number of surviving islets, is significantly lower.

3.2. Transplantation of small syngeneic graft from immune-deficient mice results in impairments in graft acceptance and glucose tolerance capacity

To further assess the capacity of islets isolated from immune-deficient mice to survive and function in an immune competent syngeneic mouse, WT mice received transplants of 100 or 50 islets from either WT or SCID mice and their blood glucose levels and glucose tolerance capacity were evaluated. As predicted by Ecoimmunity, glucose homeostasis correlated to the number of pancreatic islets transplanted. Mice transplanted with 100 islets displayed delayed establishment of normoglycemia (<250 mg/dl). These mice achieved normoglycemic levels at 20 days post transplantation, suggesting that β cells display a capacity to proliferate within transplanted islets [19]. Interestingly, mice transplanted with 50 SCID islets displayed significantly higher glucose levels throughout the entire period of the experiment (Fig. 2a, $p<0.001$ repeated measures ANOVA). These mice

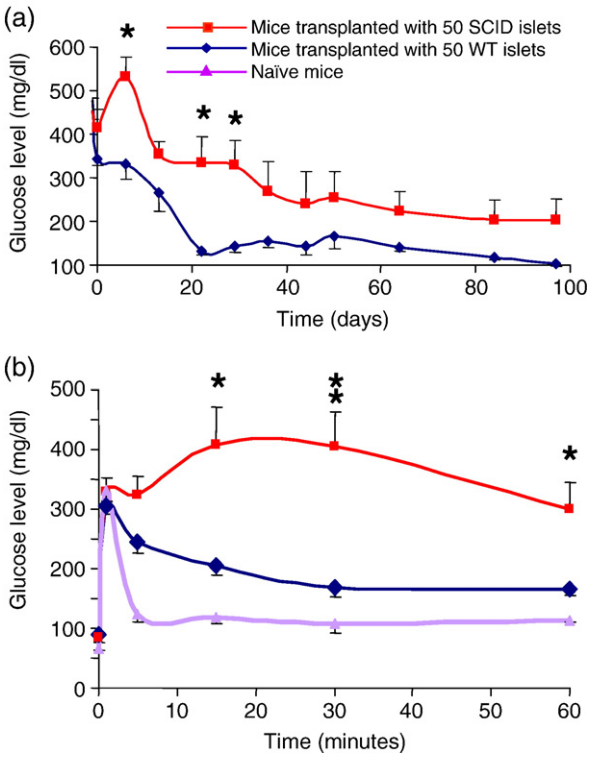

Fig. 2. Transplantation of small syngeneic graft from immune-deficient mice results in impairments in graft acceptance and glucose tolerance capacity. WT mice were transplanted with 50 syngeneic islets isolated from either WT ($n=4$) or SCID mice ($n=4$). (a) Glucose levels were determined at indicated time points. (b) Intravenous glucose tolerance test was performed 60 days post transplantation (*$p<0.05$, **$p<0.01$; two-tailed t-test).

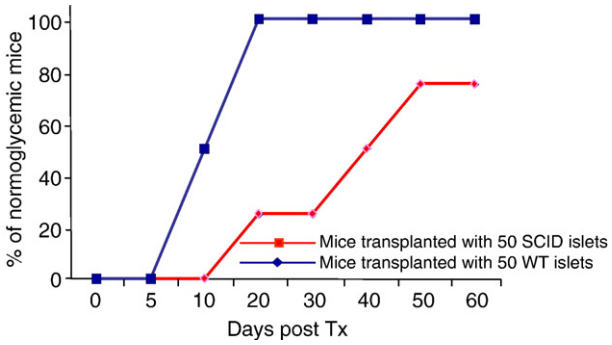

Fig. 3. Significantly delayed normoglycemia in mice transplanted with SCID islets. WT mice were transplanted with 50 syngeneic islets isolated from either WT ($n=4$) or SCID mice ($n=4$). Glucose levels were measured at indicated time points and the establishment of normoglycemia was determined (>250 mg/dl). The difference in day of graft acceptance between the two groups is statistically significant ($p<0.05$; two-tailed t-test).

achieved normoglycemic levels only at day 40 post transplantation compared with normoglycemia at day 10 in littermates transplanted with WT islets (Fig. 2a). Accordingly, the glucose tolerance test performed 60 days after transplantation revealed a significant impairment in glucose tolerance in these mice (Fig. 2b; $p<0.001$ ANOVA). Clearly (Fig. 3) the animals that received transplants of WT islets achieved glucose homeostasis more quickly than those that received SCID islets.

4. Discussion

This set of experiments demonstrates the relevance of the origin of a graft, from an immune-deficient or immune competent donor, to graft survival and function. Grafts that originate from WT and SCID animals could survive differently due to hypothetical foreignness or functional weakness of the transplanted SCID islets (both originating from the RAG mutation). We suggest that if so, 'foreignness' would imply a complete rejection of the tissue, rather than partial rejection—as observed in all mice. Weakness also disagrees with the kinetics of the deficiency. A genetically originated weakness of the SCID islets would be reflected in a continuous loss of islets—unless a process of adaptation had taken place.

In accordance with the view of Ecoimmunity, we therefore suggest that these results indicate that shortly after organ transplantation, a process of tissue adaptation by phenotype plasticity and host immunity-mediated selection takes place. Our data suggest that SCID islets are less adapted to survive and function in the presence of normal immune activity once transplanted into the WT environment. The immune response mounted toward SCID islets results in damage and loss of some β cells, while other cells adapt to avoid the immune challenge and survive. The elimination of islet tissue was more evident in animals transplanted with smaller grafts since these mice were left with the lowest number of surviving functional β cells.

Dor and colleagues [19] have demonstrated that pre-existing β cells, rather than pluripotent stem cells, are the major source of new β cells during adult life and after pancreatectomy in mice. They therefore suggest that terminally differentiated β cells retain a significant proliferative capacity *in vivo*. Moreover, these authors showed that no new islets are formed during adult life [19]. In the

context of the present work, the results of Dor et al. [18] suggest that the number of islets transplanted in each case remains constant unless immune cells eliminate some islets. On the other hand, β cells can proliferate within the limits of the islet in order to respond sufficiently to high blood glucose levels. Accordingly, transplantation of a small syngeneic islet graft (e.g., 50 islets) initially does not achieve normoglycemia; however, β cell proliferation in WT mice eventually generates sufficient insulin-producing cells to establish normoglycemia. Conversely, in mice transplanted with SCID islets, the inability of the islets to avoid immune destruction for an initial adaptation period limits the number of surviving islets and prevents the establishment of normoglycemia (Fig. 3).

The kinetics of the deficiency are also in line with this hypothesis: an initial period in which the mice have difficulty regulating glucose marks when the islets are functioning poorly due to an immune challenge. In the control group (WT islets grafted into WT mice), the mice show significantly better glucose homeostasis, since in these mice the tissue is already well adapted to cope with the local immune system.

5. Summary

This experimental system was designed to demonstrate the role of tissue maturation in the presence of a functional immune system, in the establishment of tolerance. Our findings are surprising in the context of the classical models of self/non-self. However, these results are not unique, but rather are complementary to multiple experiments performed by Triplett, Owen, and others [20–22]. In all cases the observations made are inconsistent with models in which immune tolerance is genetically predefined, or with models in which it is defined only by the immune system. Ecoimmunity suggests a new approach to the immune system–tissue interaction in which the two entities co-evolve and co-exist according to the classical ecological and Darwinian principles. Such a paradigm is capable of putting most of these observations in a coherent framework, yet additional experiments should be performed for validation. We hope that the simplicity and generalization of Ecoimmunity will stimulate experimental and theoretical scrutiny. Ecoimmunity–if correct–may spark the development of new approaches to the study of autoimmune diseases, degenerative disorders, organ transplants, and the future clinical use of engineered and cultured cells and tissues.

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