Original Article

Transplantation of Skin Grafts and Organs Infected with *Toxoplasma gondii* as a Source of Toxoplasmosis in Immunocompromised Mice

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**SUMMARY:** The possibility of *Toxoplasma gondii* infection resulting from transplantation of a skin graft and various organs has been investigated. The parasite was detected in very low numbers in all organs examined in wild-type (WT) BALB/c (B/c) mice that received skin grafts from infected interferon gamma knockout (GKO) B/c mice both with and without sulfamethoxazole treatment; all recipient mice survived. In contrast, transplantation of skin grafts from untreated infected WT B/c mice to naïve GKO B/c mice led to the death of all recipients within 20 days post-transplantation; *T. gondii* was found to be disseminated in all organs examined. Similar results were obtained after transplantation of skin from untreated and treated GKO B/c mice to naïve GKO B/c mice, whereas the recipient GKO B/c mice died within 10 days after intraperitoneal transplantation of lung, heart, brain or small intestine from infected untreated GKO B/c mice. These results indicate that skin grafts as well as various organs infected with *T. gondii* can be sources of infection in immunocompromised hosts. Toxoplasmosis should therefore be taken into consideration during organ transplantation to immunocompromised hosts.

**INTRODUCTION**

Toxoplasmosis is a self-limited infection whose symptoms are rarely present in immunocompetent hosts, whereas in immunocompromised individuals, such as transplantation recipients and patients with acquired immunodeficiency syndrome (AIDS), it may be life-threatening (1–3).

Although the continual development of new medicines means that many kinds of organ transplantation can now be carried out throughout the world, severe problems, such as post-transplantation infection, are being reported. Indeed, several patients who were seronegative for anti-toxoplasma antibodies and received hearts from seropositive donors have subsequently developed severe, life-threatening infections (4). Likewise, iatrogenic myocarditis and acute lethal toxoplasmosis have been observed in response to heart (5,6), kidney (3,7), pancreas (8), bone marrow (9,10), stem cell (11–13), liver (14,15), and lung transplantations (16).

As tachyzoites, the obligate intracellular form of the parasite, are able to invade nearly all host tissues, including skin (17,18), we suspected that skin grafts could be a source of post-transplantation infection. Thus, in the current study, we investigated the effects of the transplantation of organs, including skin, from *Toxoplasma gondii*-infected mice on the transmission of *T. gondii* to immunocompetent hosts such as wild-type (WT) BALB/c (B/c) mice and immunocompromised hosts such as interferon gamma (IFN-γ) knockout (GKO) B/c mice, since IFN-γ is an essential component of immunity against *T. gondii* infection (19). This study provides the first direct evidence that skin, as well as various other organs, can be a source of *T. gondii* infection.

**MATERIALS AND METHODS**

**Parasites:** Cysts of an avirulent Fukaya strain of *T. gondii* (20) were prepared from B10.A(4R) mice that had been infected orally with 5 cysts 6 weeks earlier, as described previously (21).

**Animals:** Eight- to 12-week-old inbred female WT B/c mice were purchased (SLC, Hamamatsu, Japan). GKO B/c mice of the same age with B/c background were kindly provided by Prof. Yoichiro Iwakura (Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, Japan). The creation of GKO B/c mice and the methods for genotyping have been described in detail elsewhere (22). Mice were handled in accordance with the guidelines of the Institutional Animal Care and Use Committee of Chiba University.

**Drug:** Sulfamethoxazole (Shionogi Co., Ltd, Osaka, Japan) was administered in drinking water at a dose of 1 mg/ml after treatment with 2 N NaOH to facilitate its dissolution in the water. The pH was then adjusted to 7 with 10 N HCl. Drug administration was initiated on day 4 post-infection (PI).

**Anesthesia:** Mice were anesthetized with sodium pentobarbital.

**Transplantation protocols:** Donor animals were infected perorally with 10 *T. gondii* cysts administered using a syringe

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fitted with a 19-gauge, round-ended needle on day 0 of the experiment.

(i) Skin grafts 10 mm² in size were obtained from untreated WT B/c mice 10 days PI and transplanted into both naïve WT B/c and GKO B/c mice.

(ii) GKO B/c mice were classified into two groups. One group remained untreated and was sacrificed at day 10 PI. The second group received sulfamethoxazole continuously from day 4 PI and mice were sacrificed at day 25 PI. Skin grafts of the same size were obtained from these groups at the days of sacrifice and transplanted into both naïve WT B/c mice and GKO B/c mice. T. gondii loads in the organs of WT B/c mice that received skin transplantation from infected untreated and treated GKO B/c mice were analyzed in skin, liver, heart, lung, kidney, brain, mesenteric lymph node (MLN), small intestine (SI), skeletal muscle, tongue, spleen, and blood 28 days after skin transplantation. T. gondii loads in skin, liver, heart, and lung of GKO B/c mice that received skin transplantation from infected untreated GKO B/c mice were analyzed 2 weeks post-transplantation.

(iii) Skin grafts of the same size were obtained from long-standing (2 months) treated GKO B/c mice and transplanted into naïve GKO B/c mice. T. gondii loads were analyzed in organs such as skin, liver, heart, lung, kidney, brain, MLN, SI, skeletal muscle, tongue, spleen, and blood 2 weeks post-transplantation.

(iv) Organs such as lung, heart, brain, and SI were obtained from untreated GKO B/c mice 10 days PI. They were fragmented and one piece (about 3 mg) was transplanted intraperitoneally into naïve GKO B/c mice (one organ per mouse). T. gondii loads were analyzed in recipient GKO B/c mouse organs such as skin, liver, heart, and lung 10 days after organ transplantation.

(v) The survival rate post-transplantation was monitored.

(vi) The day when the protozoan was first detected in the organs of recipient GKO B/c mice after skin or brain transplantations from infected untreated GKO B/c mice was estimated by checking the T. gondii load every day starting from day 4 post-transplantation.

Quantitative competitive (QC)-PCR: QC-PCR was carried out using 1 μg of genomic DNA (gDNA) from organs of WT B/c and GKO B/c mice to determine the distribution of T. gondii, as described previously (23, 24). The amplified cDNAs were separated electrophoretically on a 1% agarose gel containing ethidium bromide, and the ratio between these and the subsequently amplified competitor (T/C) surface antigen 1 (SAG1) DNA was measured using an IPLab Gel densitometer (Signal Analytical Corp., Sterling, Va., USA). The abundance of T. gondii was calculated as described previously (21, 23).

Statistics: Results of experimental studies are reported as mean ± SE. The significance of intergroup differences was determined using Student’s t test. A non-parametric Kruskal-Wallis test with Dunn’s correction was performed for multiple comparisons. The significance of differences in survival was determined using the Kaplan-Meier method. A P value of less than 0.05 was considered significant.

RESULTS

Influence of skin graft transplantation on the survival rate of recipient WT B/c mice: To determine the effects of skin transplantation on the immunocompetent recipient, skin grafts were transplanted from untreated WT B/c and from untreated and continuously treated GKO B/c mice to naïve WT B/c mice. All WT B/c mice that received skin grafts from the above-mentioned mice survived (Fig. 1A), and there was no significant difference in survival rate between the groups. We can therefore conclude that skin transplantation from untreated WT B/c mice and from untreated or treated GKO B/c mice was not lethal to WT B/c mice.

Influence of skin graft transplantation on the survival rate of recipient GKO B/c mice: To assess the effects of skin transplantation on the immunocompromised recipient, skin grafts were transplanted from untreated WT B/c and from untreated and continuously treated GKO B/c mice to naïve GKO B/c mice. All GKO B/c mice that received skin grafts from the above-mentioned mice died within 20 days after transplantation (Fig. 1B). Skin transplantation from untreated WT B/c and from untreated and treated GKO B/c mice was therefore lethal to GKO B/c mice. Although there were no significant differences in mortality between these groups, significant differences (P < 0.05) were detected between the mortality of each group of recipient WT B/c and GKO B/c mice, i.e., between the group receiving skin from untreated WT B/c mice following transplantation to WT B/c
mice and the group receiving skin from untreated WT B/c mice following transplantation to GKO B/c mice, between the group receiving skin from untreated GKO B/c mice following transplantation to WT B/c mice and the group that received skin from untreated GKO B/c mice followed by transplantation to GKO B/c mice, and between the group receiving skin from treated GKO B/c mice following transplantation to WT B/c mice and the group receiving skin from treated GKO B/c mice followed transplantation to GKO B/c mice. Thus, mortality was affected by the immune status of the recipient mice rather than by the nature of the transplanted skin.

**Influence of organ transplantation from untreated GKO B/c mice on the survival rate of recipient GKO B/c mice:** We further studied whether the intraperitoneal transplantation of organs such as lung, heart, brain, and SI from untreated GKO B/c mice influenced the survival rate of recipient GKO B/c mice and found that all recipient GKO B/c mice died within 10 days post-transplantation (Fig. 1C). These data indicate that such organ transplantation had a strong lethal effect on GKO B/c mice. There were no significant differences in survival rate between the different organ transplantation groups. However, there was a significant difference in mortality between the group receiving skin from untreated GKO B/c mice following transplantation to GKO B/c mice (Fig. 1B) and the groups receiving organs from untreated GKO B/c mice following transplantation to GKO B/c mice (Fig. 1C) ($P < 0.05$). Thus, the lethal effect of skin on GKO B/c mice was lower than that of organs such as lung, heart, brain, and SI when transplanted to GKO B/c mice.

**Influence of skin transplantation from untreated and treated GKO B/c mice on the parasitic loads of recipient WT B/c and GKO B/c mice:** In order to determine the parasitic loads after skin transplantation from untreated and treated GKO B/c mice to naïve WT B/c mice, we investigated various organs such as skin, liver, heart, lung, kidney, brain, MLN, SI, skeletal muscle, tongue, spleen, and blood of recipient WT B/c mice (Fig. 2A) 28 days after skin transplantation. The parasite proliferated slowly in all organs examined, with $T. gondii$ loads being highest in spleen, tongue, and lung, and lowest in SI, skin, blood, and kidney, after skin transplantation from untreated GKO B/c mice. A significant difference in parasite loads was detected ($P < 0.05$) between skin and spleen, kidney and spleen, SI and spleen, and spleen and blood. These data indicate that skin, kidney, SI, and blood were less affected than other organs when skin from untreated GKO B/c mice was transplanted to WT B/c mice. The $T. gondii$ loads in the organs of WT B/c mice transplanted with skin from treated GKO B/c mice were much lower (data not shown).

Furthermore, in order to determine the parasitic loads after skin transplantation from untreated GKO B/c mice to naïve GKO B/c mice, we investigated various organs such as skin, liver, heart, and lung of the recipient GKO B/c mice 2 weeks post-transplantation. The parasite was found to proliferate rapidly in the organs of GKO B/c mice (Fig. 2B), with $T. gondii$ loads being highest in heart and lowest in skin and lung. A significant difference in parasite loads was detected ($P < 0.01$) between skin and heart, liver and heart, and heart and lung. Thus, the parasite proliferated more slowly in the skin and lung than in the heart when skin from untreated GKO B/c mice was transplanted to GKO B/c mice.

**Influence of skin transplantation from long-standing (2 months) treated GKO B/c mice on the parasitic loads of recipient GKO B/c mice:** To identify the parasitic loads after skin transplantation from long-standing treated GKO B/c mice to naïve GKO B/c mice, we investigated $T. gondii$ loads in various organs such as skin, liver, heart, lung, kidney, brain, MLN, SI, skeletal muscle, tongue, spleen, and blood of the recipient GKO B/c mice 2 weeks post-transplantation (Fig. 2C). The parasite was found to proliferate rapidly in all organs examined, with the $T. gondii$ load in organs from recipient mice being highest in heart and lowest in skin, kidney, and brain. A significant difference in the parasitic loads was detected between skin and heart ($P < 0.01$), heart and kidney ($P < 0.05$), and heart and brain ($P < 0.05$). Thus, even long-term treatment with sulfamethoxazole was not sufficient to eradicate the parasite from the skin of GKO B/c mice and the cause of toxoplasmosis in skin-transplanted GKO B/c mice.

**Influence of organ transplantation from untreated GKO B/c mice on parasitic loads of recipient GKO B/c mice:** To identify any differences in parasitic loads during transplantation of organs such as brain, lung, heart, and SI, we investigated the parasitic loads in the skin, liver, heart, and lung of organ-transplanted GKO B/c mice 10 days post-transplantation (Fig. 3). The parasite proliferated rapidly in all organs examined, although significant differences were detected between many of organs examined. Thus, the $T. gondii$ load tended to be higher in heart and lung, and lower in skin, irrespective of the organ transplanted, thereby sug-
gesting that _T. gondii_ proliferates rapidly in heart and lung but only slowly in skin. In addition, the _T. gondii_ load in organs from recipient mice was highest after lung (Fig. 3B) or heart (Fig. 3C) transplantation, medium after SI transplantation (Fig. 3D), and lowest after brain (Fig. 3A) transplantation. Hence, lung and heart transplantations appear to have a significantly higher risk of causing post-transplantation iatrogenic toxoplasmosis than brain transplantation. Although skin plays a role in the transmission of toxoplasmosis, the infectious potentiality of skin was less than that of brain.

The first day of parasite detection in recipient GKO B/c mice transplanted with skin or brain from untreated GKO B/c mice: To assess whether there were any differences in the time taken for the parasite to appear after skin or brain transplantation, we investigated the day when the protozoan was first detected in organs of recipient GKO B/c mice that had been transplanted with skin or brain from untreated GKO B/c mice (Fig. 4). The protozoan was detected in SI and MLN on days 15.7 ± 1.1 and 15.2 ± 1.6, respectively, after skin transplantation, whereas it was found in the same organs on days 5.2 ± 0.5 and 5.8 ± 0.8, respectively, after brain transplantation. _T. gondii_ was observed in lung, heart, and brain on days 19.5 ± 1.3, 19.8 ± 1.1, and 20.0 ± 1.5, respectively, after skin transplantation, whereas it was detected in the same organs on days 7.2 ± 0.5, 7.0 ± 0.4, and 9.5 ± 0.2, respectively, after brain transplantation. A significant difference was therefore detected (_P_ < 0.01) between skin and brain transplantations, with _T. gondii_ being detected in the organs of GKO B/c mice significantly later after skin transplantation than after brain transplantation. Although skin plays a role in the transmission of toxoplasmosis, the infectious potentiality of skin was less than that of brain.

**DISCUSSION**

This study has shown that IFN-γ-mediated immunity plays a role in the survival rate of mice and in _T. gondii_ abundance after skin transplantation. Murray et al. (25) suggested previously that the use of GKO mice as a model for immunocompromised hosts, such as AIDS patients, makes sense in light of the fact that such individuals have a decreased ability to produce IFN-γ, a condition related to the development of opportunistic infections. Suzuki et al. (19) reported that an antibody to IFN-γ can eliminate resistance to acute toxoplasma infection in mice, thereby suggesting that this cytokine is an important mediator of host resistance to this parasite. Likewise, IFN-γ was shown to regulate _T. gondii_ loads and interconversion in the eyes of a WT B/c strain (26).

Organ transplantation is one of several routes of toxoplasmosis transmission in immunocompromised hosts such as AIDS patients (27) and in hosts undergoing immunosuppressive therapy (8). Indeed, there have been numerous reports regarding immunosuppression and acquired toxoplasmosis secondary to immunosuppression. Old age, AIDS (1,2), blood disorders, organ transplantation, connective tissue disease, and chronic immunosuppressive drug therapy have also been reported to be immunosuppressive causes in fulminant acquired toxoplasmosis (17).

The highest risk of developing toxoplasmosis in heart-transplanted patients and in a small number of other immunocompromised patients occurs in the setting of primary infection (i.e., a seronegative recipient who acquired the parasite from a seropositive donor via a graft) (28). Botterel et al. (14) and Rogers et al. (7) also reported instances of
disseminated toxoplasmosis occurring within the allograft from a seropositive donor to a seronegative recipient.

The present study found a difference in terms of survival rate and *T. gondii* load in mice receiving skin transplantation and those receiving transplantation of other organs. The reason for the longer survival and lower parasite burden after skin transplantation than that of other organs might be as follows. Skin, the body’s first line of defense against pathogens, is the largest immunological organ in which Langerhans cells/dendritic cells are abundantly distributed. These cells are representative of specialized antigen-presenting cells and play a key role in infection control (29). In addition, there is less space under the skin for pathogens to proliferate compared to the peritoneum, thus meaning that the parasite might require a longer time to multiply and disseminate throughout the body after skin transplantation. Indeed, the *T. gondii* load in the skin of WT or GKO B/c mice that had received organs, including skin, was always lower than that in other organs. Hence, lower *T. gondii* loads after skin transplantation may contribute to the extended survival time. In contrast, after organ transplantation into the peritoneum, the parasite can proliferate without space limitations and spread directly to organs that are important for survival.

In our previous article, we reported that the protozoan proliferated rapidly and disseminated to all organs, and that all GKO mice died within 10 days after cessation of short-term (11 days) sulfamethoxazole treatment (18). Furthermore, even when GKO B/c mice were treated with sulfamethoxazole continuously for a long period (2 months), *T. gondii* could be detected by QC-PCR in the brain but not in the blood or heart (30). A similar result was also reported by Suzuki et al. (31), thus proving that the brain is an organ associated with high mortality, although diagnosis was only made at autopsy in nearly half of the reported cases (32). However, according to one report, 10 out of 11 patients in whom an autemorrh diagnosis was established, and who received specific treatment, survived, thus indicating that early diagnosis and therapy are critical (33). Toxoplasmosis should therefore be regarded as a distinct possibility during organ transplantation.

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**Conflict of interest**  None to declare.

**REFERENCES**


