CUTTING EDGE

Cutting Edge: Genetic Variation Among 129 Substrains: Practical Consequences

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We designed a series of experiments to define the role of IFN-γ in cellular interactions mediating graft rejection by assessing the rejection of H-Y disparate grafts in both ligand and receptor knockout mice and their control inbred strain. In the course of these studies it became apparent that neither knockout strain is histocompatible with the putative control and that the putative control is not histocompatible with either knockout strain. In the process of deducing why this might be so, it became apparent that the putative control is not an inbred strain of mouse. Thus, in the absence of rigorous genetic control, the utility of such knockout strains of mice for assessing the effects of cytokines and receptors in transplantation and autoimmunity is limited. ***The Journal of Immunology,*** **1997, 159: 5766–5768.**

IFN-γ is a potent modulator of immune function produced by T cells and NK cells. Although it has been demonstrated that IFN-γ is required for rejection of class II MHC disparate grafts but not for rejection of class I MHC disparate grafts (1) or acute rejection of heart allografts (2), its role in the rejection of grafts requiring interactions between T cell subsets is not known. Because rejection of H-Y disparate grafts requires interaction between $CD4^+$ Th and $CD8^+$ TK cells (3), we studied the requirement for IFN-γ in the rejection of such grafts in 129 ($H\text{-}2^b$) strain mice with an interruption in the gene for IFN-γ ligand or with an interruption in the gene for IFN-γ receptor.

Materials and Methods

Animals

129/SvEvTac mice were purchased as controls from Taconic Farms (Germantown, NY). The progeny of 129/SvEvTac mice that had been blastocyst injected with AB-1 embryonic stem (ES)[2] cells containing an interrupted gene encoding IFN-γ ligand (4) and were homozygous for the interrupted gene (a gift from Dr. Edouard Cantin), and homozygous IFN-γ receptor knockout mice made with AB-1 ES cells (5) and bred onto a 129/SvEvTac background (a gift from Dr. Edouard Cantin, who obtained them from Dr. Michel Aguet) were bred in our specific pathogen-free facility.

Skin grafting

Mice were engrafted on the left flank with tail skin grafts according to an adaptation of the method of Billingham and Medawar (6). The grafts were scored daily until rejection, defined as the loss of >80% of engrafted tissue or the study end point. Mice grafted a second time were grafted above the scar from the first graft.

Results and Discussion

Experiments in which GKO and GRKO females were engrafted with control 129/SvEvTac female skin and vice versa were startling, in that both knockout strains rejected control female skin grafts with median survival times of 22 days, and control mice rejected skin from both knockout lines with a median survival time of 20 days for GKO and 23 days for GRKO (Table I and Fig. 1). These studies demonstrate that 129/SvEvTac, the purported appropriate control animal, is not histocompatible with either of the knockout strains.

Rejection times of control male grafts by GKO and GRKO females were significantly accelerated with respect to rejection time by 129 females ($p < 0.0001$, by Wilcoxon analysis; Table II and Fig. 2) and thus are consistent with responses to multiple minor disparities, but not with those to H-Y alone. In addition, the male grafts from the 129/SvEvTac mice primed the knockout mice to rapidly reject subsequently placed female grafts (Table III), providing additional support for the presence of multiple minor Ag disparities. Although these data effectively demonstrate that neither IFN-γ nor the IFN-γR is required for a primed response to minor Ags, the role of IFN-γ or IFN-γR in the rejection of H-Y disparate grafts cannot be addressed with these mice.

Studies within each knockout line, i.e., GKO male onto GKO female or GRKO male onto GRKO female, would be difficult to

Table I. *Median survival of female 129, GKO, and GRKO skin grafts on 129 female hosts and median survival of 129 female skin grafts on GKO and GRKO female hosts*[a]

Host (female)	Donor (female)	MST (days)
129 (5)	129	>70[b]
129 (5)	GKO	20
129 (5)	GRKO	23
GKO (5)	129	22
GRKO (5)	129	22

[a] MST is median survival time for the grafts. Number of mice grafted is given in parentheses. Chronic rejection is characterized by loss of hair from and shrinkage of the graft without a complete loss of the grafted tissue.
[b] One chronic rejection.

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[2] Abbreviations used in this paper: ES, embryonic stem.

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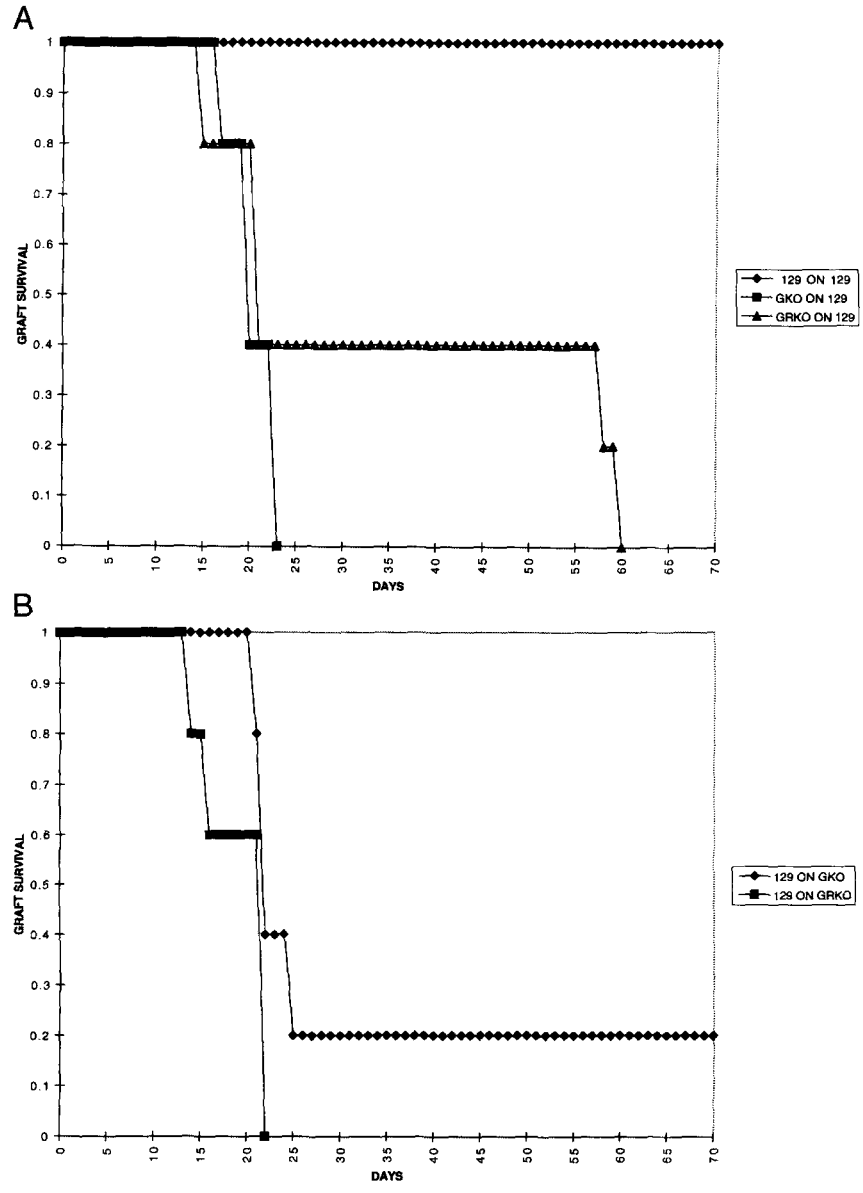

FIGURE 1. Survival curves of female skin grafts on female hosts. *A* shows graft survival curves of 129, GKO, and GRKO female skin on 129 female hosts. *B* shows graft survival curves of 129 female skin on GKO and GRKO female hosts. Wilcoxon analysis yields $p < 0.0043$, comparing graft survival of 129 female skin with that of female skin from GKO on 129 hosts, and $p < 0.0039$, comparing graft survival of 129 female skin with that of female skin from GRKO on 129 female hosts.

Table II. *Median survival times of 129 male skin grafts on GKO, GRKO, and 129 female hosts*[a]

Host (female)	Donor (male)	MST (days)
GKO (5)	129	16
GRKO (4)	129	23
129 (15)	129	31

[a] MST is median survival time. Number of mice grafted is given in parentheses.

Table III. *Rapid rejection of secondary female grafts by mice that had previously rejected primary male grafts*

Host (female)	Graft	Donor	MST (days)
GKO (5)	Primary	129 male	16
(4)	Secondary	129 female	9.5
GRKO (4)	Primary	129 male	23
(4)	Secondary	129 female	9

The study of primary graft rejection is the same study shown in Table II. MST is median survival time. Number of mice grafted is given in parentheses.

interpret. H-Y expression has not been demonstrated to be equivalent in the knockout and control mice. Additional questions arise concerning the ability of the grafted tissue from knockout mice to modulate MHC expression in the graft itself in the absence of IFN-γ or IFN-γR.

These data show that GKO mice generated by injection of AB-1 ES cells into 129/SvEvTac blastocysts are not histocompatible with 129/SvEvTac mice, and GRKO mice bred onto a 129/SvEvTac background are not histocompatible with 129/SvEvTac mice despite the Tac subline having been generated from 129/SvEv sublines.

The histocompatibility differences between the knockout mice and controls may be due to genetic differences in the ES cells and/or in the 129 line itself. Indeed, although AB-1 ES cells are $Gpi1^c$, and thus match 129/SvEvTac at this allele (7), minor histocompatibility loci closely linked with this allele may be disparate, as may other minor histocompatibility loci. As reported (7),

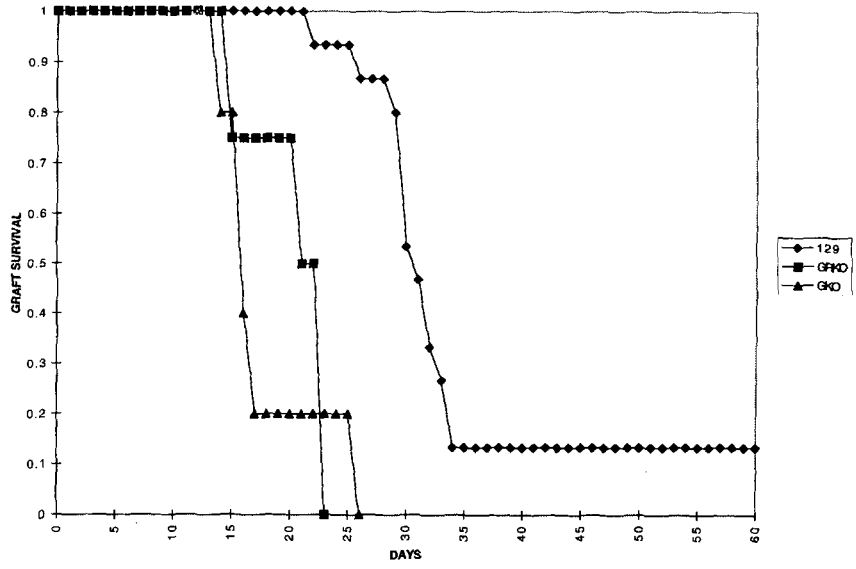

FIGURE 2. Survival curves of 129 male skin on 129 and knockout mice hosts. Wilcoxon analysis yields $p < 0.0001$, comparing graft survival on either knockout with that on control 129 hosts.

there are five simple sequence length polymorphism marker discrepancies between AB-1 ES cells and 129/SvEvTac of the 86 simple sequence length polymorphisms randomly selected and examined. Thanks to the report by Simpson et al., better genetic matches can now be selected between ES cells and inbred strains.

Moreover, genetic heterogeneity is likely within the 129/SvEvTac line itself. Founders for this colony were declared at F8 of brother × sister matings of two sublines of 129/SvEv, one of which was $Gpi1^a$ and the other of which was $Gpi1^c$ (Taconic catalogue). They are currently at F16 and therefore are not inbred. Consistent with this is our observation of the chronic rejection of a female 129/SvEvTac graft by a female littermate (Table I). To complicate matters further, some knockout colonies were established at earlier stages of brother × sister matings. Indeed, GKO mice were established in 129/SvEvTac before attainment of homozygosity at Gpi1 (E. Cantin, unpublished observation).

The report by Simpson et al. highlighted the enormous genetic variation among 129 substrains of mice. However, a few crucial issues were not resolved. First, how was $Gpi1^c$ introduced into the 129 line? Although the reported origin of the $Gpi1^c$ locus was a cross of 101 × 129 (7), 101 is described as $Gpi1^a$ (8). The $Gpi1^c$ locus is not present in any inbred strain (8), but has been reported in wild mice (9). At any rate, the introduction of this allele into 129/SvEv did not generate a strain congenic to 129/SvEv, because $Pgm1^b$ was introduced at the same time. This locus is on chromosome 5, and thus is unlinked to Gpi1, and is absent in all 129 substrains except for 129/SvEvTac and 129/SvEv-Gpi-1^c-$Hprt^{b\text{-}m2}$@J (JR2027) (7). Given this, histocompatibility would not have been expected between 129/SvEvTac and 129/SvEv.

The second major issue for clarification concerns the origin of the AB-1 line itself. In Simpson's paper, AB-1 and AB 2.1 are shown as being derived from 129/SvEvBrd-$Hprt^{b\text{-}m2}$, which is indicated as bearing $Gpi1^a$. Indeed, AB 2.1 is $Gpi1^a$, but, as noted above, AB-1 is $Gpi1^c$. Thus, the origin of these widely used ES cell lines requires further elucidation.

We concur with Simpson's admonitions on founding knockout sublines, and we wish to point out that additional nongenetic discrepancies between knockout and parental strain mice that may affect immune responses or tumor frequency could result from the standard method of producing transgenic or knockout mice by implantation of blastocysts into foster mothers, usually F1 animals. If these foster parents are from strains that transmit retroviruses vertically to nursing pups, their female pups would continue to transmit virus vertically. The presence of such retroviruses could affect the immune responses of mice by deletion of TCR subsets or be tumorigenic. Minimally, any fostering should be reported in the characterization of the engineered mouse.

Although the genetic heterogeneity that exists in the 129 substrains of mice may not play a crucial role in some types of studies, for studies of transplantation immunology and autoimmunity, genetic heterogeneity wreaks havoc. Our studies certainly illustrate that the important concerns about heterogeneity are more than theoretical.

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