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Trace Antibody Production by *scid* Mice

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Origin and History

SCID is an acronym for severe combined immunodeficiency. Animals, including humans, with this inherited disease typically lack functional B and T lymphocytes but retain normal natural killer cell (NK) activity.

The *scid* mutation in mice was discovered in 1983 in a colony of congenic BALB/c mice (C.BKa-*Igh*^{b}/IcrSz) and was determined to be an autosomal recessive. The mutation itself is a single nucleotide substitution in the *Prkdc* gene, located on chromosome 16. *Prkdc* codes for a catalytic subunit of a DNA-activated protein kinase. This DNA-activated protein kinase anneals the ends of the DNA, which is cut and rearranged (coding joint formation) as part of the process of producing antigen-specific receptors. Signal joint formation, however, occurs normally in *scid* mice. The *scid* mutation results in a premature stop codon that prevents translation of 83 amino acids at the C terminus of the protein, therefore substantially reducing DNA-PK activity. This prevents differentiation of lymphoid stem cells into more mature components of the immune system. The myeloid cell lineage is not affected by this mutation, hence the normal NK and granulocyte activity in these mice.

To inhibit the NK cell activity that could diminish tumor growth, *scid* mice were crossed with mice carrying another recessive mutation, this one in the *Lyst* gene. This mutation is known as *bg* (or beige), named for the color of the mice with this mutation, and was first described in mice in 1957 at Oak Ridge National Laboratories. *Lyst* is a gene coding for a lysosomal trafficking regulator protein. Defects in this gene result in the accumulation of giant lysosomal granules in tissues with granule-containing cells. Abnormal cells may be found in the liver, kidney, pancreas, thyroid, glandular ductal epithelium, retinal pigmented epithelium and type II pneumocytes. Importantly, mast cells, lymphocytes and granulocytes also contain these defective granules, which result in a decrease in function of the affected cells. This problem with lysosomes also leads to defective platelet formation, so beige mice have a prolonged clotting time. NK cells from beige mice have decreased endogenous cytotoxic activity, and beige mice also exhibit defective cytotoxic T cell and cytotoxic antibody responses, rendering them more permissive to the growth of both syngeneic and allogeneic tumor cells.

Purposes of *scid* Mice

scid mice are used at some institutions to investigate the immune system. However, the great majority are used as recipients of xenografted tissue. As a result, desirable criteria for a *scid* mouse are acceptance and growth of xenografts, consistent performance across groups and over time, and the easy evaluation of the implanted xenograft. *scid* mice are reported to accept xenografts more easily than athymic nude mice, probably due to the more profound immune dysfunction. As noted earlier, NK function is normal in *scid* mice, although NK regulation is abnormal (*scid*-beige have NK deficiencies). Inflammatory stimuli result in prolonged NK increases. NK cells play a role with the establishment of some xenografts, so using *scid*-bg mice is sometimes helpful.

Quality Control of *scid* Mice

An ongoing but poorly understood concern with using *scid* mice is the potential for “leakiness.” Leakiness is generally defined as increased serum immunoglobulin (Ig), although some functional T cells are also generated. The incidence of leakiness is difficult to compare across literature reports or vendors because there are no standard criteria for the leakiness threshold, the age at which leakiness is determined or what immunoglobin class(es) should be measured.

Leakiness determination varies by the measured Ig class (IgG, IgM or total Ig), and the threshold for calling an animal “leaky” varies by producer and researcher. Dr. Melvin Bosma, the discoverer of the *scid* mouse and first to publish on it, placed the leaky threshold at >50 μg/ml of all classes of Ig, measured at 100-200 days of age. Some producers only measure IgG, considering it more indicative of a mature antibody response,

whereas IgM is often produced early in an antibody response and may be less indicative of an antigen-specific response. In immunocompetent mice *in vivo*, IgM averages approximately 1,000 µg/ml, or about 5% of the 20,000 µg/ml (2 g/dl) total serum immunoglobulin. In contrast, the level of total serum immunoglobulin in a leaky *scid* mouse is usually 50-100 µg/ml, or less than 1% of that in immunocompetent mice. In addition, the Ig produced is often monoclonal or oligoclonal, rather than polyclonal, as it would be in an immunocompetent mouse. The lack of a broad polyclonal production of immunoglobulin in leaky mice suggests that the immunoglobulin is not targeted against specific antigens, but rather results from uncontrolled production by a few B cell clones. In addition, the small amount of immunoglobulin raises questions as to its biological significance, especially regarding suppression of xenograft growth, a function in which cell-mediated immunity is generally considered to be more important than humoral immunity.

The molecular basis of leakiness in *scid* mice is not well-characterized. The two possibilities generally considered for the induction of leakiness in *scid* mice, however, are genetic and environmental. Dr. Bosma claims there is no genetic basis. When he carried out selective breeding of the original *scid* mice, he did not show any effects of lineage. However, other *scid* mouse producers claim that when the *scid* mutation is placed on different background strains, there are different leakiness rates, which would seem to support a genetic basis. Unfortunately, no discussion is presented regarding the role of numerous co-variables associated with the genetic manipulation, including rederivation and microbial status. In addition, N-values of the studies are not given, and there has been no longitudinal follow-up described in these studies, so it is not clear if the change in the rate of leakiness observed initially was sustained over subsequent generations. Leakiness increases with age, and animals that are non-leaky at six weeks of age may be leaky by six months of age.

Environmental differences seem to be more likely to result in increased leakiness. This would be consistent with Dr. Bosma's observations on SPF vs. non-SPF *scid* mice and their level of leakiness. A higher level of stimulus of host defense systems, such as through pattern-recognition receptors, would result in the greater attempted activation of the immune system. Since the *Prkdc* gene product is present, just not fully functional, perhaps some cells are able to mature. The nature of the mutation is that coding joint formation occurs "poorly, if at all," but signal joint activity is normal, via *Rag1* and *Rag2* pathways. There is also support in the literature for the role of the animal's microbial milieu in leakiness development, and this is consistent with Charles River's experience.

The main question is whether leakiness hinders xenograft studies. For leakiness to have an effect on xenograft studies, the leakiness would have to correlate with restoration of immune functions. Are the T- and B-cell populations functional (vs. non-specific) in a leaky *scid* mouse? Dr. Bosma examined leaky CB17 *scid* mice in 1988 and found that they had no response to mitogens and no Ly-5+ cells in the spleen, indicating that animals were not able to respond properly to immune stimuli.

Do xenografts fare poorly in leaky *scid* mice? In the same study described above, Dr. Bosma also found that leaky *scid* mice had a higher rejection rate for allografts, although the immunoglobulin level and health status of the mice is not clearly defined in the study. In contrast, in 1993 Dr. Kollman performed follow-up work on *scid* and *bg-nu-xid* mice. There was no difference between leaky (50 µg/ml) and non-leaky *scid* in implantation rates, and no leakiness was induced by xenograft implantation. These findings suggest that not only were immunoglobulin levels approximately 1/400 of normal inconsequential, but that the mice were unable to respond to antigens in foreign cells with an antibody response. No further reports in the literature correlate xenograft growth with leakiness in *scid* mice.

Summary

The presence of trace amounts of immunoglobulin in the serum of a subset of *scid* mice (i.e., leakiness) has been recognized for more than 25 years and has raised questions among oncologists using mice. However, the molecular basis for the leakiness is not understood, nor is there scientific support for functional consequences of leakiness, as immunoglobulin levels in leaky mice are not only generally less than 1% of normal, but the immunoglobulins that are present lack the predominantly IgG and polyclonal profile expected with a normal mature immune response.

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