Agammaglobulinemia and *Staphylococcus aureus* Botryomycosis in a Cohort of Related Sentinel Swiss Webster Mice

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Sentinel mouse seroconversion to infectious agents is critical for laboratory animal facility disease monitoring. We report spontaneous emergence of non-sex-linked agammaglobulinemia with B-cell deficiency and cutaneous *Staphylococcus aureus* granulomatosis (botryomycosis) in a cohort of related Swiss Webster sentinel mice. Our experience reinforces the importance of immunocompetency validation in surveillance programs.

Immunocompetence of sentinel mice is an integral component of laboratory animal facility disease-monitoring programs. If immunity is compromised, surveillance mice may fail to detect infectious agents. This can have a serious impact on animal health and on the integrity of scientific data. Here we report the spontaneous emergence of non-sex-linked agammaglobulinemia with B-cell deficiency and frequent *Staphylococcus aureus* pyogranulomas (botryomycosis) in a cohort of sentinel Swiss Webster mice intended for disease surveillance or used as embryo transfer recipients in our transgenic facility. The condition first manifested as an unusual cluster of cutaneous botryomycosis over an 11-month period, prompting further investigation and the discovery of an underlying B-cell deficiency and agammaglobulinemia. Records indicated that all affected mice were descended from eight breeding pairs purchased from a single vendor. Depopulation and replacement with new sentinels eliminated the problem. Our experience underscores the importance of periodic validation of immunocompetence in surveillance animals, which we have incorporated into our standard operating procedures (5, 6). All protocols described in this report were in compliance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals (14) and approved by the Massachusetts Institute of Technology Committee on Animal Care.

**Clinical history.** Over an 11-month period, 28 Swiss Webster mice were submitted for necropsy with a history of abscesses or ulcerative skin lesions over the muzzle, limbs, and/or inguinal region. A standard workup for murine viruses and parasites was negative in all animals. This incidence of serious skin disease far exceeded background levels in our mouse facility, prompting the search for an underlying cause.

**Gross pathology.** Affected mice demonstrated cutaneous erythema or ulceration and epidermal thickening and often had disfiguring abscesses with tan-to-yellow-green purulent material on cut surfaces, most often on the muzzle or limbs (Fig. 1A). Others presented with a predominantly ulcerative form of dermatitis concentrated in the inguinal region. Generalized alopecia suggestive of the athymic nude mouse phenotype was not apparent (8).

**Histopathology.** Cutaneous abscesses were characterized histologically by multifocal-to-coalescing pyogranulomatous furunculosis and cellulitis with intralesional cocci circumscribed by a radiating band of hyaline eosinophilic material (Splendore-Hoeppli phenomenon; botryomycosis) (Fig. 1B). Ulcerative dermatitis in mice without discrete abscesses also frequently demonstrated a botryoid pattern. Thymus and lymph nodes lacked follicular organization, instead demonstrating uniformly prominent reticulal stroma, immature mononuclear leukocytes, and widespread apoptosis (Fig. 1C). Splenic histology demonstrated marked extramedullary hematopoiesis and myelopoiesis of the red pulp and an absence of well-formed parieto-portal lymphatic sheaths and lymphoepithelial structures in the white pulp. A morphological diagnosis of moderate-to-severe multilobular lymphopenia was assigned in all affected cases.

**Immunohistochemistry.** Cutaneous granulomas, thymus, lymph node, and spleen were labeled with antibodies to identify T cells (CD3), B cells (CD45R/B220), macrophages (F4/80), and neutrophils (myeloperoxidase [MPO]). Skin granulomas contained a central core of CD3+ T cells immediately surrounding granules of basophilic cocci bounded by Splendore-Hoeppli material (Fig. 1C). T cells in turn were bounded and infiltrated by F4/80+ macrophages and MPO+ neutrophils. B220+ B cells were extremely rare. The thymus was filled with T cells without any discernible follicular organization or corticomedullary junction (Fig. 1C). There were isolated pockets of B cells but no macrophages or granulocytes. Lymph nodes also lacked corticofollicular organization and exhibited pronounced extramedullary myelopoiesis characterized by abundant immature monocyte/macrophage and polymorphonuclear cells. The same lack of lymphoid organization with increased myelopoiesis was evident in the spleen, along with erythropoiesis.

**Complete blood count.** Peripheral lymphocyte counts of B-cell-deficient mice were compared with two different control groups descended from the same set of breeding pairs: (i) mice with a history of skin lesions and normal lymphoid organ histomorphology; and (ii) sentinel mice submitted for scheduled necropsy with no abnormalities of the skin or lymphoid organs.

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The mean circulating lymphocyte count of B-cell-deficient mice was \((3.1 \pm 0.46) \times 10^3/\mu l\). This value was significantly lower by the unpaired Student \(t\) test \((P < 0.05)\) (Prism software; GraphPad, San Diego, CA) than that of mice with skin lesions and normal lymphoid histology \((5.76 \times 10^3/\mu l \pm 0.41 \times 10^3/\mu l)\) or control mice with no abnormalities \((6.16 \times 10^3/\mu l \pm 0.42 \times 10^3/\mu l)\) (Fig. 2). Although we were unable to determine the identity of circulating lymphocytes, based on immunohistochemistry we attributed the decreased circulating lymphocyte count in affected animals to a severe depletion of B cells.

**Aerobic culture.** Nares and trachea in all mice, and skin lesions in selected mice with abscesses, were flushed with trypsic soy broth, plated onto sheep's blood, MacConkey agar, and chocolate agar (Remel Inc., Lenexa, KS), and aerobically cultured. Positive cultures were Gram stained and colonies were characterized by coagulase testing and API Staph kit (bioMerieux Industry, Hazelwood, MO) for gram-positive organ-
though we were unable to confirm this.) Horizontal lines indicate lymphocytes in agammaglobulinemic mice were probably T cells, although mean serum IgG levels (first column) versus variable but detectable IgG levels for all mice with normal lymphoid organ histology (second column) (P < 0.001). B-cell-deficient agammaglobulinemic mice also had significantly lower circulating lymphocyte counts (third column) than did immunocompetent mice (fourth column) (P < 0.01). (Circulating lymphocytes in agammaglobulinemic mice were probably T cells, although we were unable to confirm this.) Horizontal lines indicate means.

Serum gammaglobulin. Total serum gammaglobulin levels were measured using the mouse serum immunoglobulin G (IgG) enzyme-linked immunosorbent assay kit (Alpha Diagnostic International, San Antonio, TX). All B-cell-deficient mice had undetectable serum IgG (<2.4 mg/ml) and were defined as agammaglobulinemic. In contrast, mean serum IgG in lymphocompetent control mice with skin lesions was 6.76 mg/ml, and the level for healthy controls was 5.37 mg/ml (both P values were <0.001 versus B-cell-deficient mice; the Student t test) (Fig. 2). Moreover, there were significant associations between cutaneous botryomycosis, histological B-cell deficiency, and agammaglobulinemia (all P values were <0.001; Fisher’s exact test). In contrast, there was no association between the recovery of S. aureus from airways or skin and any histological or blood parameter measured (data not shown).

Sentinel mouse immunocompetence is critical to the success of surveillance programs that rely on seroconversion for the detection of murine pathogens. Whereas sporadic cases of S. aureus botryomycosis are not unusual in our colony and others, the occurrence of so many cases over 11 months significantly exceeded our historical incidence, prompting additional investigation. Strong associations were found between botryomyocosis, histological B-cell deficiency, circulating lymphopenia, and agammaglobulinemia. The identification by immunohistochemistry of T cells but not B cells in lymphoid organs was consistent with the decreased circulating lymphocyte counts and agammaglobulinemia. A review of clinical record searches ascertained that the lineage of all agammaglobulinemic mice could be traced to eight Swiss Webster breeding pairs purchased from a single vendor. We concluded that the disease was heritable, although we did not pursue a specific genetic defect. Because there was no sex predilection, it was unlikely to be analogous to human X-linked agammaglobulinemia due to a defect in Bruton’s tyrosine kinase gene. However, there are many forms of human agammaglobulinemia with no sex disparity, resulting from mutations to a wide variety of chromosomes.

The location and histological character of botryomycosis in affected mice was consistent with the introduction of S. aureus through bite wounds (limbs) and barbering (muzzle). Abe et al. described the experimental inoculation of mouse skin with S. aureus in which hair follicles were invaded within 12 h of exposure, while Akiyama et al. described clusters of S. aureus colonies and formation of a biofilm in the dermal and subcutaneous skin of mice treated with the immunosuppressive drug cyclophosphamide prior to bacterial inoculation (1, 3). S. aureus is an organism often found in the skin flora of healthy humans. Nagase et al. concluded that S. aureus was not a typical member of laboratory mouse skin flora (9), although the organism was readily cultured from both healthy and ill animals in our facility. A report of an outbreak of S. aureus-related facial and mandibular abscesses within a colony of outbred BVSV mice was attributed to a human carrier (7). S. aureus-related botryoid abscesses have been reported with high frequency in athymic nude mice (12). Direct human contact and contaminated medical devices are important sources of transmission of this and other opportunistic microorganisms in hospital settings (15). It is interesting that the botryoid lesions in affected mice exhibited the classical Splendore-Hoeppli phenomenon, characterized by a hyaline rim of smooth and club-shaped cosinophilic material surrounding bacterial colonies. Splendore-Hoeppli material purportedly is comprised of a mixture of degenerate cell debris and host Igs. However, affected mice in our study had no detectable serum IgG, suggesting that Igs may be dispensable to this process.

Murine models of combined B- and T-cell immunodeficiency, including SCID-, Rag-1-, and Rag-2-deficient mice, are well-known and widely used in research. Conversely, mouse models of pure B-cell deficiency are less pervasive. Murine models of spontaneous B-cell deficiency and hypogammaglobulinemia include A/WnSynJ, XID, and μMrtm1 mice, while examples of mutations resulting in abnormal or failed organogenesis of Peyer’s patches and myeloid dendritic cells include defects in nuclear factors such as NF-κB1, NF-κB2, and Bcl-3 (10). Spontaneous hypogammaglobulinemia and B-cell deficiency in A/WnSynJ mice and B-cell-activating factor-deficient (BAFF−/−) mice are the result of receptor mutations in the tumor necrosis factor receptor superfamily and cause maturational defects and premature apoptosis of primary and transitional B cells (4, 11). Unlike the spontaneous disease
with no sex predilection we have described here, the XID mouse model provides a model of human X-linked agammaglobulinemia due to a defect in Bruton’s tyrosine kinase gene. This spontaneous mutation affects the signal transduction of B cells and results in maturational arrest, profoundly decreased peripheral B cells, hypogammaglobulinemia, and hypoplastic lymphoid tissue (13). The role of B cells also extends to immunomodulation, as Akhiani et al. (2) demonstrated with Helicobacter pylori-infected wild-type and μMtk−/− mice. Mice with the mutation have defects in the μmt heavy chain, resulting in a maturational block of pre-B lymphocytes. The study examined H. pylori gastric colonization and the subsequent onset of gastritis and showed that while initial colonization density was lower in μMtk−/− mice, more-severe gastric inflammation with eosinophilia and CD4+ T cells developed in the absence of B cells and antibodies (2).

In summary, we report the spontaneous emergence of agammaglobulinemia with B-cell deficiency and cutaneous botryomycosis in a cohort of Swiss Webster mice. Although an outbreeding regimen was followed at our facility, all affected animals were traced to eight original breeding pairs purchased from a single vendor, suggesting a heritable defect. Therefore, the immunocompetence of outbred mice maintained as sentinels cannot be presumed. Depopulation of the affected group of animals combined with the introduction of new sentinels from another vendor has resulted in a return of skin disease incidence in our facility to historic background levels. As a result of this experience, we have incorporated periodic testing of total serum IgG as an adjunct to histology and complete blood count in order to validate the immunocompetence of sentinel mice in our laboratory animal surveillance program.

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