liver (*11*) is required before these tissues can grow, expand, or regenerate.

In the Paris *et al.* work, a single large dose of radiation caused endothelial cell apoptosis in the intestinal mucosa. If a similar effect is seen with fractionated radiotherapy (a more clinically relevant treatment), this could have profound implications for cancer therapy. For example, if the microvascular endothelial cell is the principal target of radiotherapy and damage to the epithelial stem cell is a secondary event, this relationship may also hold for endothelial cells and the tumor cells they support. This scenario would explain the synergistic effects obtained when radiotherapy is combined with antiangiogenic therapy (*12, 13*). Even if the endothelial cell response is only a component of the tumor response to radiation, attacking both compartments is a logical therapeutic strategy.

A poorly understood feature of radiotherapy treatment is that some tumors are very radiosensitive in vivo (for example, Hodgkin's lymphoma) and others are very radioresistant (for example, glioblastoma), whereas in vitro these tumors have similar or overlapping radiosensitivities (*14*). In vitro, tumor cells are the only target and ionizing radiation directly damages their DNA, inducing them to undergo apoptosis. However, in vivo, there are a multitude of supporting cells (including endothelial cells) that may be more sensitive to ionizing radiation than tumor cells, which then die not because of DNA damage but because they require endothelial cell support. The Paris *et al.* report prepares the stage for studying the effects of radiation on microvascular endothelial cells recruited to the tumor bed during angiogenesis. It may be possible to modify the radiosensitivity of a tumor by increasing or decreasing circulating endothelial inhibitors or stimulators, thereby making the tumor microvasculature more radiosensitive. If further evidence supports the idea that the microvascular endothelial cell is the principal target of ionizing radiation, as indicated by the provocative results of Paris *et al.*, then treating tumors first with angiogenesis inhibitors may sensitize the tumors to ionizing radiation, allowing lower radiation doses to be used.

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PERSPECTIVES: IMMUNOLOGY

Tampering with the Immune System

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The immune system must maintain a delicate balance between the positive signals that activate lymphocytes and the negative signals that dampen inappropriate immune responses. If this balance is upset, the immune system either does not respond to pathogens or responds inappropriately, resulting in autoimmune disease. Antigens, cytokines, and death-inducing members of the tumor necrosis factor (TNF) family (*1, 2*) all contribute to homeostatic regulation of the immune system. Several recent reports, including one by Lu and Lemke (*3*) on page 306 of this issue, now implicate the Tyro-3 family of receptor tyrosine kinases as important players in immune regulation. The Tyro-3 family (Tyro-3, Axl, and Mer) may fine-tune the immune response by modulating the activity of macrophages and other antigen-presenting cells (APCs) that present antigen to T and B lymphocytes (*3–5*).

The Tyro-3 receptor tyrosine kinases have an extracellular region composed of two immunoglobulin-like domains and two fibronectin-like domains, and an intracellular kinase domain that contains a distinctive Lys-Trp-Ile-Ala-Ile-Glu-Ser motif [reviewed in (*6*)]. These receptors are overexpressed by many tumors and can transform (immortalize) cultured cells in vitro, suggesting that they provide positive growth-promoting signals to cells. They activate Src family kinases and signaling pathways downstream of Grb2 (an adapter protein in the Ras pathway), promoting cell proliferation and protecting against programmed cell death (apoptosis). The ligands for these receptors, protein S (an anticoagulant) and Gas6, are expressed by many cell types and share similarities with sex hormone–binding globulin. Although the Tyro-3 receptors and their ligands have been well characterized, the signaling pathways that they activate are poorly understood.

In previous work, Lu and Lemke (*7*) reported that triple mutant mice lacking the Tyro-3, Axl, and Mer receptors (TAM) contained large numbers of apoptotic cells in many of their tissues, resulting in reproductive, neural, and immune abnormalities. They now report that these TAM-deficient mice also bear the hallmark features of autoimmunity: increased numbers of activated lymphocytes, lymphocytic invasion of multiple organs, increased serum autoantibodies, and deposition of immune complexes in tissues (*3*). The presence of anti-phospholipid antibodies and cerebral hemorrhages in TAM-deficient animals is reminiscent of the human antiphospholipid syndrome characterized by an increased risk of thrombosis, stroke, and miscarriage. This human autoimmune thrombolytic disorder is associated with a decrease in serum protein S, which is presumed to be a secondary event related to the hypercoagulation deficit. The Lu and Lemke study, however, raises the intriguing possibility that a decrease in serum protein S may contribute directly to the autoimmune process through a decrease in Tyro-3 receptor activation (*3*).

How do the Tyro-3, Axl, and Mer receptors contribute to regulation of the immune system? Tyro-3 receptors are not expressed by quiescent lymphocytes, but are expressed by many other cell types including APCs (see the figure). Both macrophages and CD11b-positive APCs from TAM-deficient mice become hyperactivated when stimulated with bacterial lipopolysaccharide (LPS) in vitro (*3*). Macrophages freshly isolated from TAM-deficient animals have increased expression of major histocompatibility complex class II molecules and produce elevated amounts of interleukin-12, suggesting that

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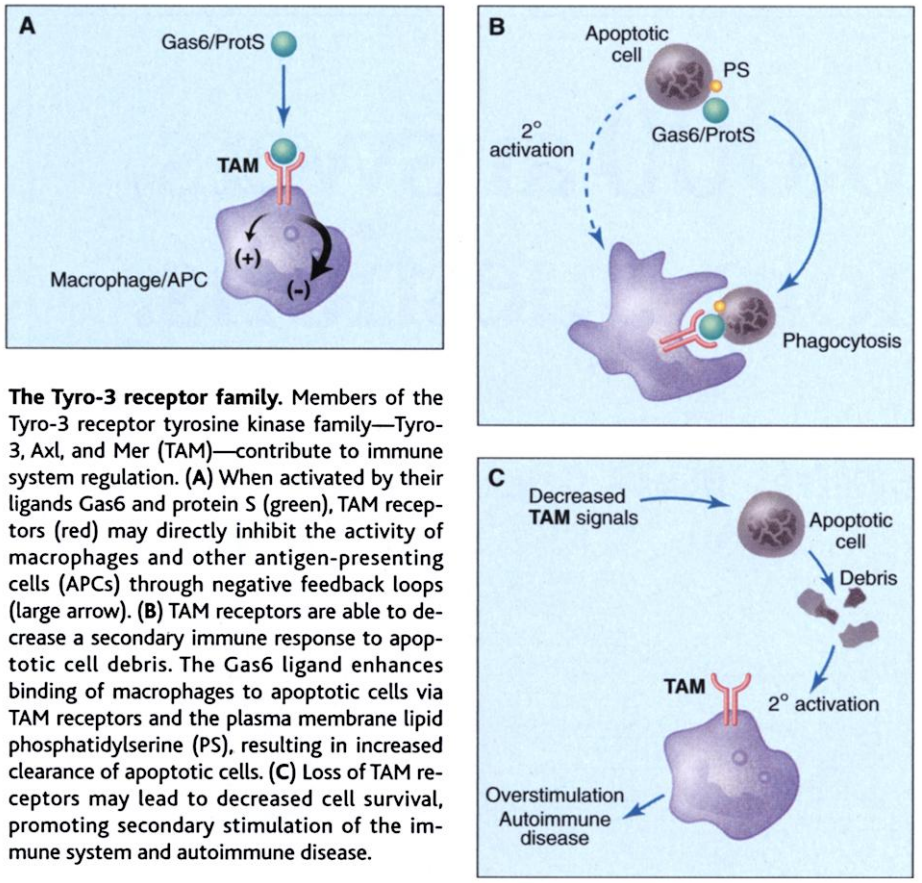

The Tyro-3 receptor family. Members of the Tyro-3 receptor tyrosine kinase family—Tyro-3, Axl, and Mer (TAM)—contribute to immune system regulation. **(A)** When activated by their ligands Gas6 and protein S (green), TAM receptors (red) may directly inhibit the activity of macrophages and other antigen-presenting cells (APCs) through negative feedback loops (large arrow). **(B)** TAM receptors are able to decrease a secondary immune response to apoptotic cell debris. The Gas6 ligand enhances binding of macrophages to apoptotic cells via TAM receptors and the plasma membrane lipid phosphatidylserine (PS), resulting in increased clearance of apoptotic cells. **(C)** Loss of TAM receptors may lead to decreased cell survival, promoting secondary stimulation of the immune system and autoimmune disease.

they may have been preactivated before removal from the mice. Wild-type T and B lymphocytes transferred to TAM-deficient animals proliferate inappropriately, indicating that some feature of the TAM-deficient animals must be leading to premature lymphocyte activation.

The authors propose that APCs lacking TAM receptors are hyperactivated and that they enhance the activation of lymphocytes. Support for this notion comes from the study of mice deficient in the Mer receptor. These animals are much more susceptible than wild-type mice to LPS-induced septic shock because their macrophages overproduce TNF-α, a factor known to be involved in septic shock (*3*). These findings suggest that Tyro-3, Axl, and Mer may normally block the activation of macrophages and other APCs (see the figure).

Most of the available evidence indicates that Tyro-3 receptors convey growth-promoting and pro-survival signals to cells. Many tyrosine kinase receptors, however, are also able to activate negative feedback loops that attenuate the positive signaling pathways that they have activated. The hyperactivated state of APCs and lymphocytes from TAM-deficient mice suggests that the loss of negative signals from the Tyro-3, Axl, and Mer receptors has more profound effects than the loss of the positive growth-promoting signals. Which negative signaling pathways might be operating downstream of the Tyro-3 receptors? Lu and Lemke argue that the Tyro-3 receptors contain a sequence that resembles an immunoreceptor tyrosine-based inhibitory motif (ITIM) (*3*). This motif, identified within the inhibitory receptors of immune cells, recruits tyrosine phosphatases (enzymes that attenuate signaling pathways by removing phosphate groups from signaling components). However, although the motifs are similar, the sequence in Tyro-3 receptors probably does not have ITIM activity because it has a charged residue in the –2 position.

Recent work by Matsushima's group adds another intriguing twist to the plot so far. These authors found that mice deficient in the Mer receptor were unable to clear immature T cells in the thymus (thymocytes) that had been induced to undergo apoptosis by dexamethasone (*5*). The researchers chose to investigate clearance of apoptotic cells in these mice because they knew that Gas6 promotes binding of macrophages to phosphatidylserine, a plasma membrane phospholipid involved in the recognition of apoptotic cells (see the figure). Thymocytes from wild-type and Mer-deficient mice die at similar rates in response to dexamethasone in vitro. However, in vivo, mutant mice retain elevated numbers of apoptotic thymocytes because their macrophages are unable to ingest (phagocytose) the dying cells. Mutations in the human and rat *mer* gene cause retinal degeneration associated with defective phagocytosis of dying retinal photoreceptor cells by macrophages (*8*). Matsushima's group points out that an increase in the number of apoptotic cells and a decrease in their clearance could result in increased amounts of cellular debris. Such debris could aberrantly stimulate the immune system, perhaps accounting for the hyperactivated state of APCs and lymphocytes noted by Lu and Lemke in their TAM-deficient mice. Intriguingly, Lu and colleagues previously reported that their TAM-deficient mice have increased numbers of apoptotic cells in many different tissues (*7*). However, altered clearance of apoptotic cells may not be the only source of dead cells in these mice. Given that Tyro-3 receptors are known to operate in cell survival pathways, it is possible that both altered cell survival and decreased phagocytosis of apoptotic cells contribute to the lymphoproliferative and autoimmune features of the TAM-deficient animals. Either way, an increase in dead cells and cellular debris may result in secondary activation of the immune system and hyperactivation of APCs and lymphocytes (see the figure).

Thanks to the description of the TAM-deficient and Mer-deficient mice, it is now clear that Tyro-3 receptors and their ligands contribute to the normal regulation of the immune system. Inappropriate regulation of these receptors and their signaling pathways could lead to alterations in immune homeostasis and the development of autoimmunity. The observation that disruption of the Tyro-3, Axl, and Mer receptors leads to abnormal activation of immune cells underscores the complex nature of signal transduction, where loss of signals may lead to unexpected outcomes.

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