Activation of Tyrosine Kinase Signal Pathways by Radiation and Oxidative Stress

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Most research on ionizing radiation, ultraviolet radiation, and H_2O_2 exposure has focused on the well-known ability of such agents to damage cellular components, particularly DNA. However, recent studies have shown that these events also act directly on components of tyrosine kinase signal transduction pathways, resulting in their activation. Cells use these types of pathways to transmit signals from surface receptors to the nucleus in response to a wide variety of stimuli, ranging from hormones and growth factors such as insulin, erythropoietin, and epidermal growth factor to antigen stimulation of lymphocytes. We propose that cellular responses to radiation and oxidative stress involve the active process of tyrosine kinase signal transduction, in addition to damage to DNA and other cellular components, leading to the activation of transcription factors and the subsequent induction of gene expression. The ability of radiation and oxidative stress to bypass control by normal ligands to act on receptors and their signal transduction pathways offers a new perspective on the ways in which organisms can respond to stress. (Trends Endocrinol Metab 1994;5:383–388)

A cell's surface receptors receive multiple stimuli from a variety of hormones and growth factors and in turn transmit this information to the nucleus via multistep signal cascades that can amplify as well as transduce the signal. Rather than being isolated independent systems, these signal pathways are interconnected, forming complex networks that integrate the stimuli to give a biologic response, usually involving changes in gene expression, as recently reviewed for G-protein-coupled receptor systems (Schöfl et al. 1994). However, many hormones and growth factors such as insulin, erythropoietin, and epidermal growth factor (EGF) act via tyrosine-kinase-dependent mechanisms. Recent studies in our laboratories and elsewhere indicate that radiation and oxidative stress may strongly affect these interconnected signal pathways, in addition to normal regulation by authentic ligands. We have used lymphocytes as a model system to explore these events for two reasons. First, tyrosine phosphorylation is the central regulatory pathway in lymphocytes, with antigen recognition being the primary signaling event, although many cytokines and cell surface molecules also act in achieving a productive response. Second, lymphocytes are highly sensitive to oxidative stress (Ames et al. 1993, El-Hag et al. 1986) and radiation, providing a biologic context for the significance of these effects. In this review, we relate these findings to signal pathways with many features in common to those utilized by hormones, cytokines, and growth factors of interest to the endocrinologist.

T cells are the primary regulatory cells of the immune system, with $CD4^+$ cells providing help in the form of cytokines and cell surface ligands to monocytes and to B cells responsible for antibody production (Paul and Seder 1994), and $CD8^+$ cells having cytotoxic function. T cells are exposed to oxidative stress in the form of H_2O_2 at sites of inflammation due to the action of neutrophils and macrophages, and this has been shown to be a means by which neutrophils exert immunosuppressive effects (El-Hag et al. 1986, El-Hag and Clark, 1987). Two enzymes that catabolize H_2O_2, catalase and thioredoxin, act as autocrine growth factors for T cells, further demonstrating the sensitivity of T cells to exogenous H_2O_2 (Sandstrom and Buttke 1993, Yodai and Uchiyama 1993). The inflammatory cytokine tumor necrosis factor α (TNF α) also stimulates production of H_2O_2 and lowers cellular glutathione levels (Klebanoff et al. 1986, Staal et al. 1990).

Lymphocytes are also exposed to oxidative stress in the course of treatments with ionizing radiation and ultraviolet irradiation. Lymphocytes are extremely sensitive to ionizing radiation, and this sensitivity has been used to advantage in the treatment of leukemia and lymphoma. Ultraviolet (UV) irradiation has promoted graft acceptance and inhibited graft-versus-host disease in a variety of transplantation models, and these effects are ascribed to the high degree of sensitivity of lymphocytes, particularly T cells, to UV (Pamphilon et al. 1991). Most research on the effects of UV and ionizing radiation has focused on DNA damage. Similarly, H_2O_2 can cause oxidative damage to cellular components, and thus many responses have been considered in terms of loss of function. Recently, however, we and others have found that oxidative stress in the forms of H_2O_2, ionizing radiation, or UV radiation can actually activate key tyrosine kinase signal transduction pathways.

• Tyrosine Kinase Signal Transduction Pathways

Tyrosine phosphorylation is the earliest detected response to stimulation of antigen receptors of T (June et al. 1990a) and B (Gold et al. 1990) cells. The mobilization of intracellular free calcium ions is an essential component of a wide variety of signal transduction pathways, including both guanine nucleotide-binding regulatory protein (G protein)-type path-

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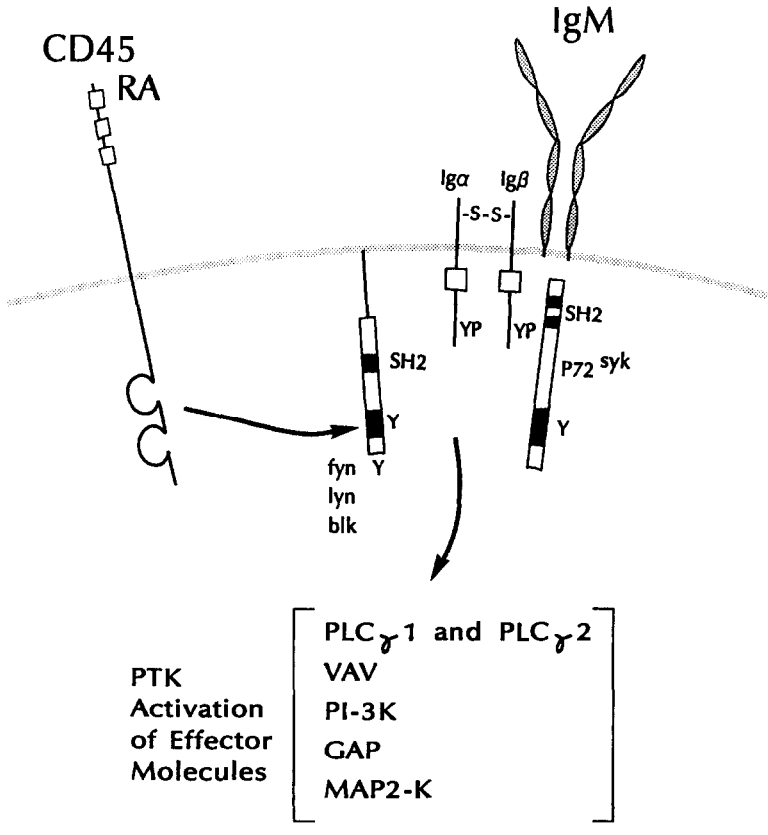

Figure 1. Tyrosine kinase signal transduction pathways in B cells. PTK (protein tyrosine kinase) activation of the effector molecules occurs either by direct phosphorylation or indirectly through PTK initiation of signal cascades. Tyrosine phosphorylation sites are indicated by Y, and phosphotyrosine is indicated by YP. The effector molecules include PLC (phospholipase C), vav, which acts as a guanine nucleotide exchange factor for Ras or related proteins, PI-3K (phosphatidylinositol 3-kinase), GAP (GTPase-activating protein for Ras), and MAP2-K (mitogen-activated protein kinase). The RA isoform of the CD45 phosphotyrosine phosphatase is shown in this example.

ways, such as for adrenergic receptors and tyrosine-kinase-dependent pathways, such as for insulin and platelet-derived growth factor (PDGF) receptors. In T and B cells responding to antigen, this type of Ca^{2+} signal in response to antigen and productive lymphocyte activation requires tyrosine kinase activity (Lane et al. 1991, June et al. 1990b). Signal transduction by antigen receptors in lymphocytes requires two types of tyrosine kinases, the Src-family kinases and the Syk-family kinases, as well as the transmembrane phosphotyrosine phosphatase CD45 (see Weiss and Littman 1994 for review). Src-family kinases are 50- to 60-kD proteins that associate with the inner surface of the plasma membrane and the cytoplasmic tails of a variety of cell surface receptors. Some Src-family kinases are present in many cell types, where they play essential roles in cellular responses to growth factors such as EGF and PDGF, but other family members are restricted to hematopoietic cell lineages (Bolen et al. 1992). A key regulatory mechanism for Src kinases is a regulatory tyrosine in the C-terminal tail of the proteins that maintains the kinases in an inactive state as long as that tyrosine is phosphorylated. Mutations in this region can cause constitutive activation of the kinase that may lead to oncogenic transformation of the cell. Syk-family kinases are 70- to 72-kD cytoplasmic proteins restricted to hematopoietic cells. These kinases lack the C-terminal regulatory tyrosine and appear to be regulated by the action of Src-family kinases as described later.

B cells recognize antigens directly by receptors consisting of surface antibody (surface immunoglobulin or sIg) and the associated MB1 (Igα) and IgB chains (Figure 1). Receptor binding of antigen triggers the activation of Src-family kinases such as Fyn, Lyn, and Blk, as well as the Syk kinase. T cells have a distinct set of molecules in the T-cell receptor (TCR) that are analogous to those in the B-cell receptor, and the basic signaling pathways are similar as well (Figure 2). T cells recognize peptide antigens after processing by antigen presenting cells, which display for the T cells the processed peptides bound to major histocompatibility complex (MHC). The TCR binds the antigen–MHC complex. CD4 binds directly to MHC II, stabilizing the interaction with the TCR and also enhancing TCR signaling with the aid of its associated Lck kinase. CD8 has a similar interaction with MHC I. The TCR includes the variant α and B chains that recognize the antigen, the invariant δ, ε, and γ chains comprising CD3, plus a dimer consisting of ζ or ζ plus η chains. The Src-family kinases Lck and Fyn, plus the Syk-related kinase ZAP-70, play central roles in TCR signaling. ZAP-70 associates with tyrosine phosphorylated ζ and ε chains during signaling. The activation of these kinases in B and T cells results in the phosphorylation and activation of many substrates common to growth-factor-signaling pathways (Figures 1 and 2). Expression of the phosphotyrosine phosphatase CD45 is essential for lymphocyte tyrosine kinase signaling. This is due at least in part to the role of CD45 in maintaining the C-terminal regulatory tyrosines on the Src-family kinases in a dephosphorylated state so that the kinases are active.

- **Ionizing Radiation, Apoptosis, and NF-κB Activation**

Recent work has suggested that tyrosine phosphorylation plays a major role in lymphocyte responses to ionizing radiation. Exposure of B-cell lines to therapeutically relevant doses of ionizing radiation (50–400 cGy) results in the rapid induction of cellular protein tyrosine phosphorylation (Uckun et al. 1992). Pretreatment of the cells with tyrosine kinase inhibitors such as herbimycin A and genistein blocked this induction, whereas the phosphotyrosine phosphatase inhibitor orthovanadate augmented the tyrosine phosphorylation response, suggesting that this response involved the activation of tyrosine kinases and that phosphotyrosine phosphatases might limit the response. Kinase assays demonstrated that tyrosine kinases were indeed activated by radiation (Uckun et

al. 1992). Direct assays with anti-Lck antibodies have indicated that irradiation of B-lineage leukemia cells rapidly but transiently activates the Lck tyrosine kinase (Waddick et al. 1993). Furthermore, treatment with genistein inhibited radiation-induced apoptosis and clonogenic cell death, whereas vanadate sensitized the cells to ionizing radiation (Uckun et al. 1992). Apoptosis is distinct from necrosis in that apoptotic cells follow a program of cell death that often involves new gene expression. These effects on radiation-induced apoptosis are significant because the induction of apoptosis and clonogenic cell death is the goal of radiation therapy for leukemia and lymphoma. These results suggest that the sensitivity of lymphocytes to low doses of radiation is due to activation of tyrosine kinase pathways leading to apoptosis.

The transcription factors c-jun and NF-κB are not only among the first to be activated after antigen stimulation of lymphocytes, but also play an important role in the induction of gene expression by radiation. Recent work indicates that there is a common underlying mechanism for these seemingly diverse effects. Extensive studies have shown that free-radical scavengers such as N-acetyl cysteine block the activation of NF-κB by a wide range of stimuli, including TNFα, interleukin-1 (IL-1), phorbol esters, ionizing radiation, H_2O_2, lipopolysaccharide, and calcium ionophores (Schreck et al. 1991). These results indicate that reactive oxygen intermediates act as key messengers in these processes. Tyrosine kinase inhibitors such as genistein and herbimycin block the activation of NF-κB by H_2O_2, suggesting that the reactive oxygen species use tyrosine kinases to activate NF-κB (Schieven et al. 1993c). Treatment of cells with ionizing radiation activates protein kinase C, leading to the induction of c-*jun* and the activation of NF-κB (Sherman et al. 1990, Hallahan et al. 1991, Brach et al. 1991). However, tyrosine kinase inhibitors such as herbimycin block the induction by ionizing radiation of protein kinase C and NF-κB (Uckun et al. 1993), as well as the induction of c-*jun* (Chae et al. 1993). Taken together, these results suggest that both radiation and biologic stimuli induce and activate these transcription factors via a common signal pathway involving tyrosine phosphorylation. Our results on the receptor dependence of UV induction of NF-κB support this hypothesis.

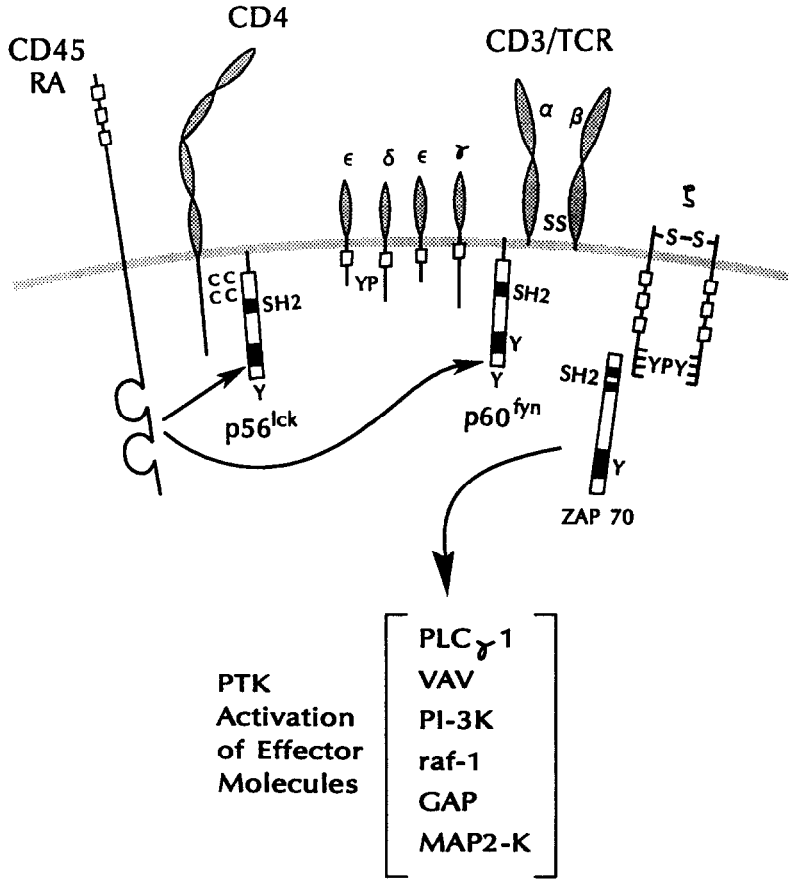

Figure 2. Tyrosine kinase signal transduction pathways in T cells. The signal pathway is similar to that of B cells. Raf-1 is a serine/threonine kinase regulated by Ras in the signal cascade leading to MAP kinase activation.

• Tyrosine Kinases Responsive to H_2O_2

In order to understand better how reactive oxygen species engage tyrosine kinase signal pathways, we have used H_2O_2 as a pharmacologic agent. H_2O_2 is an insulin mimetic agent that increases glucose transport and oxidation, stimulates lipogenesis, stimulates glycogen synthase, and increases tyrosine phosphorylation of the insulin receptor and other cellular proteins (Heffetz et al. 1990). Treatment of B cells with H_2O_2 induced Ca^{2+} signals in a dose-dependent manner (Schieven et al. 1993a). The basis for these signals was revealed by the finding that H_2O_2 induced inositol-1,4,5-trisphosphate (IP_3) production within 10 sec of exposure (Schieven et al. 1993c). IP_3 binds to Ca^{2+} channels, opening the channels and giving rise to Ca^{2+} mobilization (Berridge and Irvine 1984). Tyrosine kinase inhibitors blocked both the IP_3 production and the calcium signals (Schieven et al. 1993a and c). Taken together, these results indicated that H_2O_2 treatment resulted in Ca^{2+} signals via a process similar to that which occurs for biologic stimulation.

H_2O_2 treatment of B cells resulted in cellular tyrosine phosphorylation within seconds of treatment as well (Schieven et al. 1993a). The overall pattern of tyrosine phosphorylation was strikingly similar to that observed for biologic stimulation via surface immunoglobulin (sIg) as well as for UV (Schieven and Ledbetter 1993), suggesting an underlying common mechanism. Although cross-linking sIg activates Src-family kinases (Burkhardt et al. 1991), Lck, Fyn, and Lyn activity did not detectably increase after treatment with H_2O_2 (Schieven et al. 1993a). In contrast, as illustrated in Figure 3, Syk tyrosine kinase activity is highly responsive to treatment of cells with H_2O_2, giving a very similar dose response to H_2O_2 as was observed for Ca^{2+} signals and cellular tyrosine phosphorylation

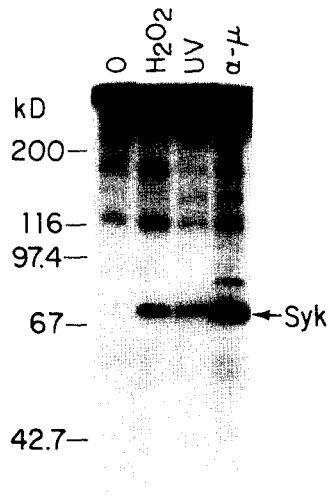

Figure 3. Activation of Syk tyrosine kinase activity by UV, H_2O_2, and sIg cross-linking. Ramos B cells were untreated (0), treated with 2400 J/m^2 UVC, with 7.5 mM H_2O_2, or with anti-μ (anti-IgM) F(ab')$_2$, and Syk immune complex kinase assays were then performed as previously described (Schieven et al. 1993a). Proteins labeled with ^{32}P in the kinase reaction were resolved by gel electrophoresis and detected by autoradiography. The autophosphorylated Syk protein is indicated by the arrow.

(Schieven et al. 1993a). Further kinase assays indicated that Syk was the primary H_2O_2 responsive tyrosine kinase in the B cells. The responsiveness of Syk to H_2O_2, UV, and sIg stimulation (anti-μ) illustrated in Figure 3 provides a common mechanism for the similar patterns of tyrosine phosphorylation observed for these diverse stimuli. T cells can be even more highly responsive to H_2O_2 in terms of cellular tyrosine phosphorylation and Ca^{2+} signals than B cells (G. Schieven unpublished results). We have found that the Syk-related tyrosine kinase ZAP-70 is highly responsive to H_2O_2 in these cells, associating with the ζ and ε chains of the TCR, its physiologically relevant partners (Schieven et al. 1993a and 1994). The ZAP-70 response to H_2O_2 was dependent on TCR expression, but did not require CD45, in contrast to normal TCR signaling.

Why are Syk and ZAP-70 so responsive to H_2O_2? The key to answering this question may lie in the observation that direct treatment of immunoprecipitated Syk with H_2O_2 has little effect, nor does direct treatment of activated Syk with reducing agents (Schieven et al. 1993a). Thus Syk-family kinases do not appear to be directly regulated by oxidation. Instead, kinase activation occurs only when intact cells are exposed to oxidative stress, indicating that cellular components that regulate Syk-family kinases are the targets. Some of the targets may include phosphotyrosine-phosphatases. H_2O_2 inhibits phosphotyrosine phosphatases (Hecht and Zick 1992), and the mechanism of ZAP-70 regulation (reviewed in Weiss and Littman 1994) would support the hypothesis that phosphatase inhibition could lead to ZAP-70 activation. ZAP-70 contains two SH2 domains. The binding of SH2 domains of proteins to proteins with phosphotyrosine residues contained in specific amino acid sequences recognized by the SH2 domains permits the assembly of signaling complexes that are integral to the productive stimulation of cells by a wide variety of hormones and growth factors such as insulin and EGF (Songyang et al. 1994). Activation of ZAP-70 involves the binding of the tandem SH2 domains to tyrosine phosphorylated ζ or ε chains of the TCR, and phosphorylation of ZAP-70 on tyrosine as a result of its intrinsic kinase activity or by the action of another kinase such as Lck. Inhibition of phosphatase activity could permit accumulation of tyrosine phosphorylated ζ and ε chains in the presence of basal levels of cellular tyrosine kinase activity, leading to ZAP-70 activation. Although Syk activation is not as well understood, this kinase also has tandem SH2 domains and could be regulated by similar mechanisms. because phosphatases can regulate signaling by receptor tyrosine kinases, including the insulin receptor, and because SH2 interactions are essential components of signaling by these receptors for a wide variety of hormones and growth factors, this mechanism is likely to affect many pathways in a variety of cell types.

- **Ultraviolet Radiation (UV)**

The findings described earlier for ionizing radiation led us to examine the effects of UV on lymphocyte signaling. UVB (302 nm) and UVC (254 nm) irradiation strongly induced tyrosine phosphorylation in both B cells and T cells, whereas UVA irradiation had little effect (Schieven et al. 1993b). Furthermore, UV strongly induced Ca^{2+} signals in T cells via tyrosine phosphorylation of phospholipase C g1 and associated proteins, but not in B cells (Schieven et al. 1993b). Similarly, in normal peripheral blood lymphocytes, both $CD4^+$ and $CD8^+$ T cells gave strong Ca^{2+} signals in response to UV, whereas other cell types were not responsive (Schieven and Ledbetter 1993). Interestingly, ionizing radiation did not result in Ca^{2+} signals (Schieven et al. 1993b). Thus although ionining radiation, UV, and H_2O_2 all can induce tyrosine phosphorylation, ionizing radiation does not induce Ca^{2+} signals, UV induces Ca^{2+} signals in T cells, and H_2O_2 induces Ca^{2+} signals in both T and B cells, suggesting that there must be differences in the signal mechanisms activated by these treatments. Recently, we have observed that the UV-induced tyrosine phosphorylation and Ca^{2+} signals in T cells are mediated via the TCR and ZAP-70, and are also regulated by the CD45 phosphotyrosine phosphatase, indicating a mechanism very similar to that employed by biologic stimulation of the TCR (Schieven et al. 1994). We also found that the activation of NF-κB by UV radiation in T cells requires expression of the TCR (Schieven et al. 1994). These UV effects are not limited to lymphocytes, but instead clearly apply to other cell types and receptor systems. UV activates NF-κB in enucleated HeLa cells, demonstrating that a mechanism other than DNA damage is responsible for the response (Devary et al. 1993). In HeLa cells, the mammalian UV response, which consists of a specific pattern of gene expression similar to that induced by many growth factors, is triggered by activation of Src-family kinases in a signaling cascade involving Ras and Raf, two signaling proteins that play essential roles in signaling by many hormone and growth-factor-specific receptor tyrosine kinases (Devary et al. 1992). This pathway appears to help cells survive radiation exposure, perhaps by inducing biosynthesis to replace cellular components damaged by the radiation (Devary et al. 1992). It has also recently been reported that the EGF receptor is activated in epidermal cells within 30–60 min of UV irradiation (Warmuth et al. 1994, Miller et al. 1994). However, we have observed EGF receptor responses within 30 s, demonstrating that UV acts

rapidly on such receptor tyrosine kinases (Schieven et al. 1994). Because receptor tyrosine kinases can activate Src kinases via SH2 interactions (Kypta et al. 1990, Koch et al. 1991), this may account for the UV activation of Src kinases reported previously. Taken together, these results suggest that UV can activate cell surface receptors that are either linked to tyrosine kinases, such as the TCR linked to ZAP-70, or that have intrinsic tyrosine kinase activity, such as the EGF receptor. This in turn appears to lead to new gene expression. The activation of such molecules by UV would provide a mechanism that would readily explain the UV induction of gene expression in a pattern overlapping with that induced by growth factors.

• Summary

Ionizing radiation, UV, and H_2O_2 have all been found to activate key tyrosine kinase signal transduction pathways in lymphocytes as well as in other cell types. In addition to causing damage to cellular components such as DNA, we propose that many cellular responses to radiation and oxidative stress are mediated via activation of signal pathways normally under biologic control. Cell surface receptors that normally respond to biologic stimulation appear to also be responsive to UV radiation, leading to activation of transcription factors that alter gene expression. Similarly, oxidative stress in the form of H_2O_2 appears to act not only on such receptors, but can also in some cases bypass the receptors to act on downstream components. The inappropriate activation of regulatory tyrosine kinase pathways is therefore likely to contribute to the high sensitivity of lymphocytes to radiation and oxidative stress. In a broader context, these findings suggest that radiation or oxidative stress might act on a variety of systems in a manner that bypasses normal endocrine control. This will be an important area for future research.

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