Gene microarray identification of redox and mitochondrial elements that control resistance or sensitivity to apoptosis

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Contributed by Leonard A. Herzenberg, December 23, 1999

Multigenic programs controlling susceptibility to apoptosis in response to ionizing radiation have not yet been defined. Here, using DNA microarrays, we show gene expression patterns in an apoptosis-sensitive and apoptosis-resistant murine B cell lymphoma model system both before and after irradiation. From the 11,000 genes interrogated by the arrays, two major patterns emerged. First, before radiation exposure the radioresistant LYar cells expressed significantly greater levels of message for several genes involved in regulating intracellular redox potential. Compared with LYas cells, LYar cells express 20- to 50-fold more mRNA for the tetraspanin CD53 and for fructose-1,6-bisphosphatase. Expression of both of these genes can lead to the increase of total cellular glutathione, which is the principle intracellular antioxidant and has been shown to inhibit many forms of apoptosis. A second pattern emerged after radiation, when the apoptosis-sensitive LYas cells induced rapid expression of a unique cluster of genes characterized by their involvement in mitochondrial electron transport. Some of these genes have been previously recognized as proapoptotic; however others, such as uncoupling protein 2, were not previously known to be apoptotic regulatory proteins. From these observations we propose that a multigenic program for sensitivity to apoptosis involves induction of transcripts for genes participating in mitochondrial uncoupling and loss of membrane potential. This program triggers mitochondrial release of apoptogenic factors and induces the ''caspase cascade.'' Conversely, cells resistant to apoptosis down-regulate these biochemical pathways, while activating pathways for establishment and maintenance of high intracellular redox potential by means of elevated glutathione.

Understanding the genetic programs that regulate the balance between apoptotic cell death and survival is critical for elucidating molecular mechanisms of pathologies as diverse as cancer and autoimmunity. The advent of gene microarrays permits the analysis of gene expression patterns for a large numbers of genes, allowing the macrodissection of molecular events during apoptosis and potentially identifying novel pathways and regulators (1). We used this technology to extend previous observations made in a B cell lymphoma model, using two cell lines that were derived from the same tumor yet have different levels of BCL-2 protein and glutathione (GSH) (LYas, lymphoma apoptosis sensitive; LYar, lymphoma apoptosis resistant) (2–5).

Sample heterogeneity inherently makes analysis of thousands of genes in large cell samples difficult. One advantage of using radiation as an inducing agent is that it is instantaneous; therefore sample heterogeneity induced by drug delivery and metabolism is eliminated. Most model systems do not lend themselves to microarray analysis of radiation-induced apoptosis, however. Unlike apoptosis induced in thymocytes or splenocytes, apoptosis that does occur in many culture systems is asynchronous, occurs relatively late (after 12–48 hr), and is incomplete (30–60% of cells undergo apoptosis), resulting in heterogeneous mRNA preparations. In contrast, the LYas/LYar culture system spans the extremes of radiation sensitivity. The LYar cells are as radioresistant as Chinese hamster ovary cells, whereas the LYas cells are as radiosensitive as ataxia–telangiectasia cells (4). Furthermore, the kinetics of apoptosis in the LYas cells are synchronous; apoptosis is not seen before 2 hr after irradiation and is virtually complete in all cells by 5 hr (4). Therefore, we used this model to generate homogeneous RNA to analyze multigenic programs mediating radiation-induced death.

Methods

Cell Culture. LYas and LYar cells are two clonal sublines obtained from an apoptotic sensitive B cell mouse lymphoma (TH-LY) and cultured as described in ref. 4.

Microarray Sample Preparation. Details for the sample preparation and microarray processing are available from Affymetrix (Santa Clara, CA) and will be described elsewhere. Briefly, total RNA was prepared from cells by using a Qiagen RNeasy midi kit. Poly(A)$^+$ RNA was prepared from total RNA by using oligo(dT) resin (Oligotex; Qiagen). Two micrograms of mRNA was used to prepare double-stranded cDNA (Superscript; GIBCO/BRL) with a T7-$(dT)_{24}$ primer containing a T7 RNA polymerase promoter site (Operon). Biotinylated complementary RNA was made from 1 μg of cDNA (Ambion T7 Megascript kit; Bio-11-CTP, Bio-16-UTP, Sigma) and then fragmented to approximately 50–100 nucleotides. Affymetrix provided the procedures for sample preparation. Ten micrograms of the *in vitro* transcripts with appropriate controls and spikes were hybridized to an Affymetrix Mu11K microarray for 16 hr at 45°C with constant rotation at 60 rpm.

Chips were washed and stained by using the EukGE-WS2 protocol on a Affymetrix fluidics station. The stain included streptavidin-phycoerythrin (10 μg/ml; Molecular Probes) and biotinylated goat anti-streptavidin (3 μg/ml; Vector Laboratories). Chips were scanned with an HP argon-ion laser confocal microscope, with a 488-nm emission and detection at 570 nm.

Data Analysis. Fluorescence intensity was measured for each chip and normalized to the average fluorescence intensity for the entire chip. The values were scaled to 150 so that all chips could be directly compared, and the data were imported into an Access

Abbreviations: GSH, glutathione; FABPs, fatty acid binding proteins; VDAC, voltage-dependent anion channel; UCP, mitochondrial uncoupling protein; GGT, γ-glutamyl transpeptidase.

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Table 1. GenBank accession number, fold change, and Entrez definition of the top 30 differentially regulated genes

	Accession no.	Fold	Entrez definition	Group
			Genes elevated in LYar cells	
1	M25324	66	Mouse peripheral lymph node-specific homing receptor (MEL-14 antigen) mRNA, complete cds	
2	**AA276043**	**56**	**Similar to gb:L10320 fructose-1,6-bisphosphatase**	1
3	**ET63206**	**45**	***Mus musculus* mRNA for fructose-1,6-bisphosphatase, partial**	1
4	Msa.1236.0	44	*Mus musculus* thymosin B4 (*Ptmb4*) gene	
5	U38252	34	*Mus musculus* FX-induced thymoma transcript (FXI-T1) mRNA, complete cds	
6	Msa.14975.0	33	Homologous to sp P17097: zinc finger protein 7	
7	Msa.43194.0	31	*Mus musculus* membrane glycoprotein gene (extracted 3′-UTR)	
8	Msa.13789.0	28	Homologous to sp P17861: X box binding protein-1 (XBP-1) (TREB5 protein)	
9	**Msa.376.0**	**27**	**Mouse adipocyte lipid binding protein gene, complete cds**	2
10	**U80819**	24	***Mus musculus* glutathione *S*-transferase homolog mRNA, complete cds**	3
11	J05118	21	Mouse mast cell carboxypeptidase A mRNA, complete cds	
12	**Z16078**	**21**	***Mus musculus* CD53 exon 7**	4
13	**Msa.23975.0**	**20**	**Homologous to sp P19112: fructose-1,6-bisphosphatase**	1
14	**Msa.32472.0**	**20**	**Homologous to sp P09467: fructose-1,6-bisphosphatase**	1
15	U67187	20	*Mus musculus* G protein signaling regulator RGS2 (*rgs2*) mRNA, complete cds	
			Genes elevated in LYas cells	
1	**X06086**	**64**	**Mouse mRNA for major excreted protein (MEP). Secreted L-cathepsin**	5
2	AF023873	45	*Mus musculus* single-minded 2 (*Sim2*) gene, exon 11 and complete cds	
3	d90362	43	Mouse mRNA for cadherin-associated protein (CAP102/α-catenin)	
4	**Msa.547.0**	**39**	**Mouse cathepsin L gene, complete cds, clones a-H-ras-1 and RIT-1**	5
5	**X53825**	**27**	***Mus musculus* mRNA for heat stable antigen**	6
6	AA407907	25	EST01752 Mouse 7.5 dpc embryo ectoplacental cone cDNA library *Mus musculus* cDNA clone C0013F05 3	
7	Msa.1760.0	24	*Mus musculus* Src-like adapter protein SLAP mRNA, complete cds	
8	**Msa.30310.0**	**21**	**Homologous to sp P06797: cathepsin L precursor (EC 3.4.22.15) (major excreted protein) (MEP)**	5
9	**Msa.453.0**	**20**	**Mouse argininosuccinate synthetase (ASS) mRNA, complete cds**	7
10	**M31690**	**19**	**Mouse argininosuccinate synthetase (ASS) mRNA, complete cds**	7
11	C76683	19	Similar to human CYP2D7AP pseudogene for cytochrome	
12	**Msa.13177.0**	**17**	**Homologous to sp P24807: signal transducer CD24 precursor (M1/69-J11D heat stable antigen)**	6
13	U72680	17	*Mus musculus* ion channel homolog RIC mRNA, complete cds	
14	Msa.7690.0	16	Homologous to sp P41732: cell surface glycoprotein A15	
15	D10849	15	Mouse mRNA for thromboxane A2 receptor, complete cds	

Fold changes in gene expression were determined by using Affymetrix software, comparing the mRNA intensity at each gene for LYar and LYas cells. In the upper 15 entries, fructose-1,6-bisphosphatase (group 1), adipocyte lipid binding protein (group 2), the glutathione *S*-transferase (group 3) previously identified in this system (ref. 2), and CD 53 (group 4) were all elevated 20- to 66-fold in the LYar cells compared with the LYas cells. In the lower 15 entries, LYas cells expressed elevated message levels for the cysteine protease L-cathepsin (group 5), heat stable antigen (group 6), and argininosuccinate synthetase (group 7) compared with LYar cells before irradiation.

(Microsoft) database with corresponding expressed sequence tags (ESTs) and gene description for analysis. For direct visualization the data were imported into JMP (SAS Institute). The clustering and display programs (http://rana.stanford.edu/software) developed by Eisen et al. (1) were also used for analysis.

Results

mRNA was collected from LYas/LYar cells before irradiation to assess initial gene expression differences. In untreated samples, the expression of approximately 750 (6.8%) genes was elevated greater than 3-fold higher in LYar cells (maximum 66-fold) compared with LYas cells. Conversely, the expression of 340 (3.1%) genes were higher in LYas cells (maximum 64-fold) (Table 1).

Since any transcriptional regulation of apoptosis-related genes should occur soon after irradiation, we analyzed gene expression patterns within 2 hr after 5 Gy of irradiation (Fig. 1). Samples were collected at 15-min increments after irradiation for the first hour and one sample at the end of the second hour. Duplicate samples were taken at 0, 30, and 60 min after irradiation. Coefficients of variance were calculated for the expression of each gene for all time points after irradiation. We found that a variance threshold of 2.5 could distinguish statistically higher or lower gene expression from random variation. After radiation treatment, 1,828 genes (14%) in LYas samples had variances greater than 2.5, and LYar samples had 2,261 genes (17%) with variances that exceeded 2.5 (Fig. 2). Of these genes, 1,644 (12%) were induced in both cell lines (radiation response), whereas 184 genes (1.4%) were uniquely induced in LYas (death response) and 617 (5%) were uniquely induced in LYar (survival response) (Fig. 2).

Pearson rank clustering (1) of differences in expression for each gene between all the samples and untreated LYas cells revealed groups of genes that had not been previously recognized to regulate apoptosis. Three distinct patterns emerged from clustering analysis: genes that are induced in both LYas and LYar cells, genes that are preferentially induced in LYar cells, and genes that are preferentially induced in LYas cells (Fig. 3).

mRNA transcripts that increased in response to radiation in both cell lines were primarily ribosome-associated genes (Fig. 3*B*). Of the remaining two patterns, many of the genes that were differentially regulated were not previously identified as regulating apoptosis.

Striking among these were alterations in genes controlling intracellular redox potential (CD53 and fructose-1,6-bisphosphatase) (Table 1), as well as genes shown to be involved in the regulation of mitochondrial function (FABPs, UCPs, and VDACs) (Table 1 and Fig. 3). While it is beyond the scope of this report to describe in detail all the genes that were differentially expressed, several of these are described below.

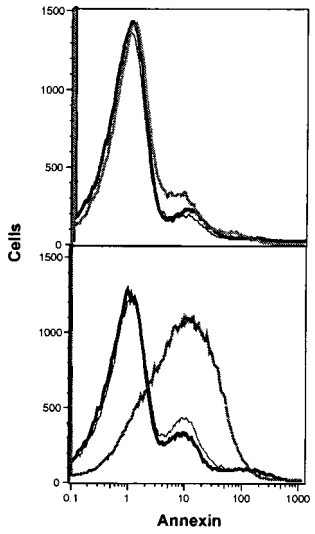

Fig. 1. Annexin staining of LYas and LYar cells after 5 Gy of irradiation. LYas and LYar cells were stained with annexin V protein at different intervals after irradiation to measure loss of membrane phosphatidylserine asymmetry, an indication of apoptosis. (*Upper*) Virtually no annexin V staining was detected in LYar cells before irradiation (thick black line), at 2 hr after irradiation (thin black line), or at 5 hr after irradiation (thick gray line). (*Lower*) A slight increase in annexin V staining could be detected in some LYas cells at 2 hr (thin black line) both with respect to LYar cells and with unirradiated LYas cells (thick black line). However, at 5 hr after irradiation (thick gray line), all the LYas cells stained positive for annexin V, compared with few LYar cells staining positive.

CD53. Analysis with gene microarrays identified the surface marker CD53 as differentially expressed to a large degree (Table 1). We confirmed this result by FACS analysis of CD53 surface expression. CD53 is a member of the TM4 (transmembrane 4) family of surface proteins (6), which are known to interact with other surface proteins; however, the binding partners are only now being identified. Recently, it was reported that CD53 associates with the GSH-metabolizing protein γ-glutamyl transpeptidase (GGT) (7), which suggested to us a mechanism for the previously unexplained observation of elevation of GSH levels in LYar cells (3, 5).

The tripeptide GSH does not cross the plasma membrane (8). However the amino acid components of GSH (the most important being cysteine) can enter the cell when membrane-bound GGT cleaves GSH to γ-glutamyl-compounds and cysteinylglycine, which is further cleaved by a membrane-bound constitutive dipeptidase into cysteine and glycine. The γ-glutamyl products of GGT and the free amino acids released by the dipeptidase can then enter the cell and contribute to the resynthesis of GSH via the GSH synthesis pathway (γ-glutamylcysteine synthetase and GSH synthetase) (9) (Fig. 4).

We previously reported that LYar cells, as well as other cells containing expression constructs for BCL-2, have significantly higher levels of intracellular GSH, particularly in the nuclei (3, 5). In these studies there were no apparent differences in the activity of the enzymes responsible for GSH biosynthesis (unpublished observations), therefore, extracellular GSH catabolism, coupled with amino acid transport, especially of cysteine, is a potential pathway for elevating intracellular levels of GSH in apoptosis resistance.

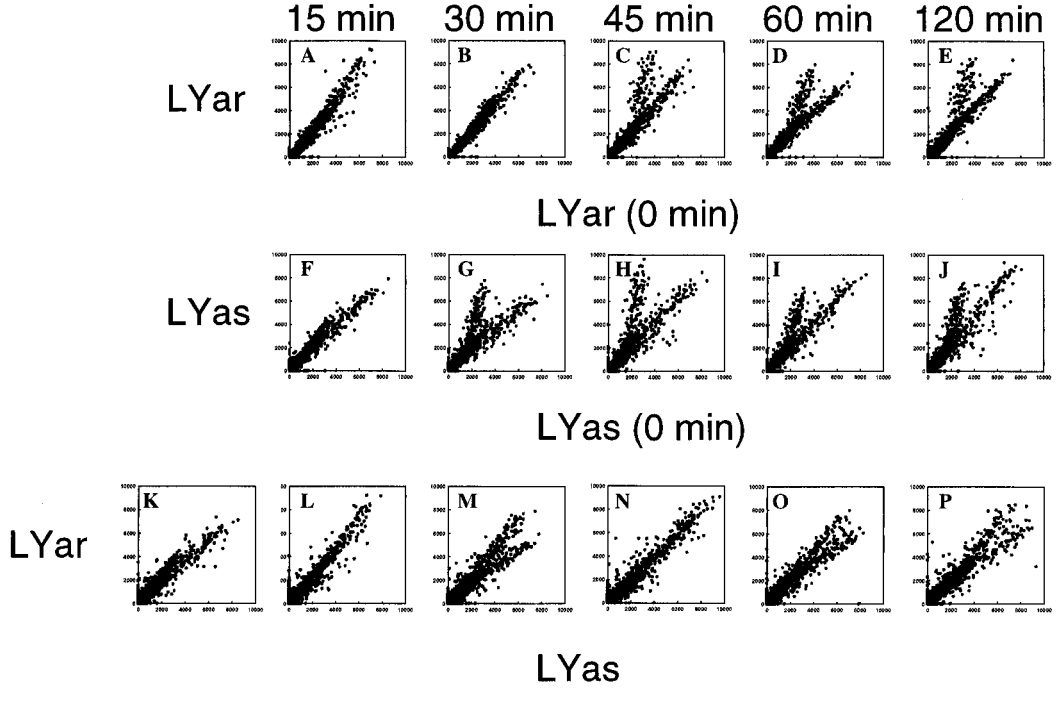

Fig. 2. Scatter plots of gene expression for LYar and LYas cells after irradiation. Gene populations became apparent after gene expression levels in unirradiated cells were plotted against irradiated cells collected at indicated times (*A–J*). Gene induction in response to radiation is visualized as a shift upward from the diagonal, while genes suppressed are seen as shifted downward. Both cell lines respond to radiation by rapidly inducing a subset of genes. While the gene induction is slightly delayed in the LYar cells, in both cell lines little gene suppression is observed. Many of the genes induced in response to radiation are induced in both cell lines. These genes are visualized as falling along the diagonal when LYar cells are plotted against LYas cells (*K–P*). Points deviating from the diagonal represent genes differentially expressed between the two cell lines. Two distinct populations (survival response versus death response) were detected within 30 min after irradiation. This is the earliest distinct measure (occurring between 15 and 30 min after irradiation) defining a death versus a survival response in these cells. All other measures of apoptosis in these cells—i.e., GSH loss, decrease in mitochondrial membrane intensity, loss of cytochrome *c*, and DNA fragmentation—occur beginning at least 1.5 hr after irradiation (3–5, 33, 34). It should be pointed out that many of these messages code for genes of unknown function, and further characterization would lead to confirmation for our proposed pathways and additional new pathways.

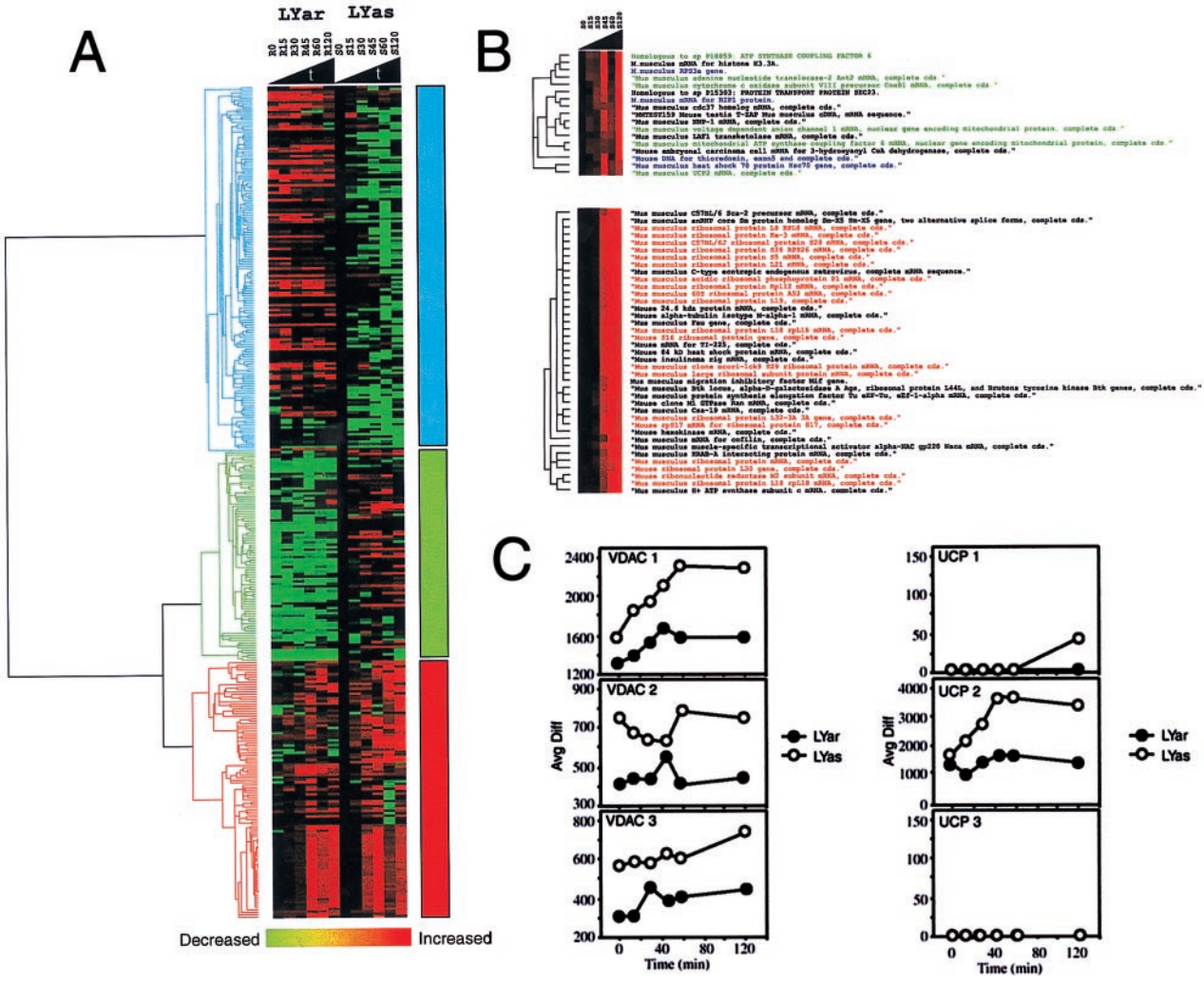

Fig. 3. Cluster Analysis of LYar and LYas response to radiation reveals mitochondrial and apoptotic gene induction. (*A*) To categorize the responses of the two cell lines to radiation, Pearson rank cluster analysis was performed on the average difference (AD, calculated by subtracting the mismatch signal from the perfect match signal) values for all 11,000 genes present on the array (1). The AD value for each gene was subtracted from the AD value for that gene in unirradiated LYas cells to cluster the genes with similar changes in expression levels. Each column presents the expression of that gene at the indicated time relative to unirradiated LYas cells. Red indicates increased expression, and green indicates decreased gene expression. Line length in the dendrogram indicates correlation, with shorter lines indicating greater correlation. Three major groups of genes emerged after radiation. Genes that were induced in both cell lines (red), genes preferentially elevated in LYar cells [fatty acid binding proteins (FABPs), CD53, and fructose-1,6-bisphosphatase] (blue), and genes elevated in LYas cells (see below) (yellow). The complete data set can be found at http://apoptosis.stanford.edu. (*B*) Radiation-induced genes expressed in LYas and LYar cells. Discrete cluster subsets from above data detailing genes induced by radiation only in LYas cells (*Upper*) and in both cell lines (*Lower*). Of the genes induced in LYas cells only, some have previously been implicated in regulating apoptosis (blue), and a subset of mitochondrial genes is also identified (green). Most of the genes induced in both cell lines are ribosomal genes. (*C*) AD time course for voltage-dependent anion channel (VDAC) and mitochondrial uncoupling protein (UCP) family members from mRNA collected at 0, 15, 30, 45, 60, and 120 min after 5 Gy of irradiation. Significant mRNA message increased only for UCP-2 in the LYas cells (*Left*). VDAC-1 is the only family member that increased dramatically (*Right*). Increased message is detected immediately after radiation exposure and plateaus at 1 hr.

Fructose-1,6-bisphosphatase. Fructose-1,6-bisphosphatase, along with a number of its homologues, was among the top 15 genes most differentially up-regulated in LYar cells compared with LYas cells (Table 1). Fructose-1,6-bisphosphatase is a major enzyme involved in gluconeogenesis (another being glucose-6-phosphatase), where it opposes the rate-limiting enzyme in glycolysis (10), phosphofructokinase, which is down-regulated in LYar cells. Generation of glucose 6-phosphate provides substrate for the pentose phosphate pathway to generate reducing equivalents (10). Enhanced activity of the pentose phosphate pathway generates a total of two NADPH equivalents via the oxidation of glucose 6-phosphate to 6-phosphoglucolactone and oxidation of 6-phosphogluconate to ribulose 5-phosphate (10). The reducing equivalents provided by NADPH, in turn, contribute to the reduction of oxidized glutathione by glutathione reductase, forming a redox cycle. Thus, the gene array data accord with previous findings that apoptosis-resistant, BCL-2-overexpressing cells have increased pools of GSH (Fig. 4) (3). Additionally, recent reports describe increased pools of NAD(P)H in Daudi cells transfected with BCL-2, which is also consistent with our findings (11).

FABPs. Comparison of LYar and LYas mRNA levels in unirradiated cells revealed that a family of FABP genes are up-regulated in LYar cells, again in the group of 15 genes most up-regulated (Table 1). These genes include transcripts for MAL-1 (the keratinocyte lipid-binding protein), adipocyte lipid-binding protein, 3T3 lipid-binding protein, and FABP, which

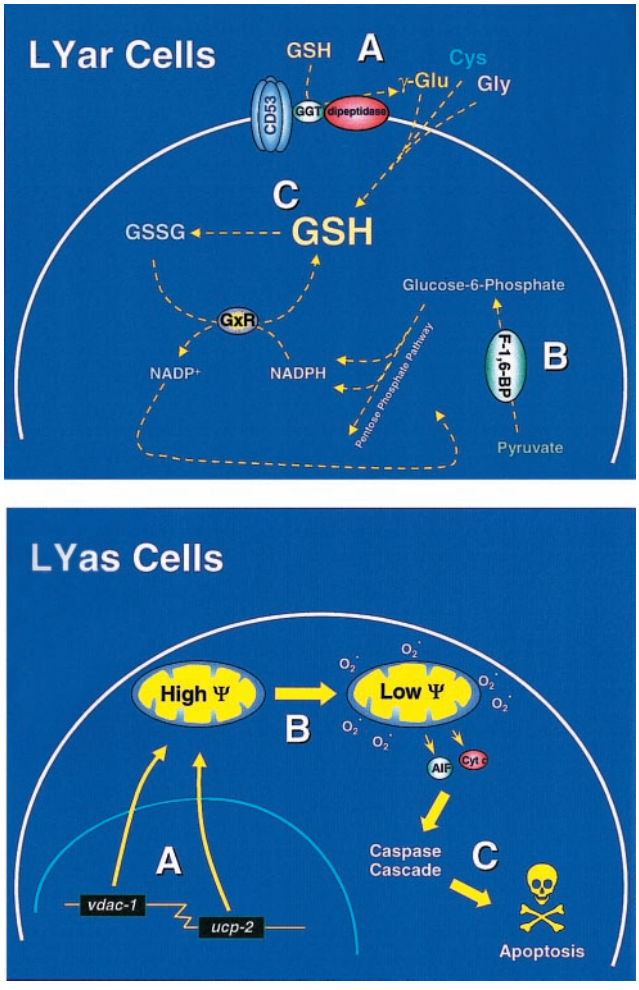

Fig. 4. Hypothetical LYar and LYas cells. From the data obtained, we have constructed a hypothetical model for the genes involved in apoptosis regulation. (*Upper*) LYar cells, which have previously been shown to have elevated GSH (C) (3), expressed message for CD53 and fructose-1,6-bisphosphatase that was not detected in the LYas cells. Both of these genes can combine to maintain elevated intracellular pools of GSH by enhancing the transport of GSH precursors into the cell (A) and keeping GSH in its reduced form by generating NADPH by enhanced gluconeogenesis and pentose phosphate pathway activity (B). GSSG, oxidized GSH; GxR, glutathione reductase. (*Lower*) In contrast, LYas cells trigger apoptosis in response to radiation by means of initiating transcription of proteins that adversely affect mitochondrial function. Radiation-induced increases in VDAC-1 and UCP-2 (A) uncouple mitochondrial electron transport and dissipate mitochondrial membrane potential (Ψ) (B), thus activating the release of apoptogenic factors. This leads to the activation of catabolic enzymes (primarily caspases), which complete the cell death process (C). AIF, apoptosis-initiating factor; Cyt *c*, cytochrome *c*.

were all detected at elevated levels in LYar cells, whereas some were not detected at all in LYas cells.

Free fatty acids are present during many phases of fat metabolism, trafficking, and signaling. In neoplastic skin cells (12), carcinomas, and gliomas (tumors especially resistant to apoptosis) (13), FABPs are elevated. Additionally, in a model of chemically initiated malignant skin tumors, differential screening of cDNA libraries identified a hyperproliferative keratin (K6) which is a member of the FABP family (14).

The increase of FABPs in tumor cells is notable because this class of proteins binds the fatty acid retinoic acid (15), which has been previously shown to function as a differentiation signal as well as an apoptotic signal in transformed cells (16). Interestingly, apoptosis-resistant (BCL-2-expressing) cells have been shown to abrogate the ability of retinoic acid to induce apoptosis (17). Exploring the direct relationship between BCL-2 and FABPs may illuminate the mechanism through which BCL-2 is able to prevent apoptosis in response to retinoid exposure.

Why are FABPs elevated in cells resistant to apoptosis induction? Increased reliance on β-oxidation of fatty acids for metabolic energy is one possibility. Alternatively, the ability of FABPs to bind, sequester, and buffer fatty acid signaling molecules, such as arachidonic acid or ceramide, may "short circuit" the effector role of these apoptotic lipids. Finally, as has been described for the nematode FABP, these proteins may act as molecular scavengers, binding toxic oxidized fatty acids generated during oxidative stress (18), thus terminating a cascade of lipid peroxidation, observed as an early step in the commitment phase of many apoptotic systems. It is interesting that increased lipid peroxidation was observed in BCL-2-knockout mice (19). The observed increased lipid peroxidation observed by Hockenbery *et al.* (19) led them to speculate that BCL-2 functioned in some manner to inhibit lipid peroxidation. While those studies were searching for a direct mechanism for BCL-2 in regulating lipid biochemistry, our results suggest that BCL-2 as a transcriptional regulator (20, 21) may function upstream of lipid peroxidation, thereby enhancing a cell's capacity to buffer and terminate these destructive pathways.

UCP. UCP-2 (and not UCP-1 or UCP-3) is elevated in LYas cells and increased significantly in response to radiation (Fig. 3*C*). UCP-1 was originally identified in brown adipose tissue and is implicated in regulating thermogenesis (22). Since the discovery of UCP-1 two additional family members have been cloned (UCP-2 and UCP-3). UCP-2 and UCP-3 have been detected in skeletal muscle and appear to regulate fatty acid metabolism (23, 24). Mitochondria are the targets of UCP function and, as their name suggests, they are involved in uncoupling electron transfer from oxidative phosphorylation. Free fatty acids are also known uncouplers of electron transport, and the release of apoptotic factors from the mitochondria has been attributed to the effect of free fatty acids on mitochondria (25). Mitochondria are the source of cytochrome *c* and apoptosis-initiating factor, both of which have been shown to be involved in the assembly of the apoptosome (with the cooperation of the ced-3 homologue APAF-1) (26, 27); specific up-regulation of message for one of the UCP family members (UCP-2) suggests that there may be another mechanism through which mitochondrial function is altered before any observable mitochondrial deregulation. UCP-2 message peaks at 1 hr, whereas no mitochondrial changes are seen until 1.5–2 hr in LYas cells. Whether UCP-2 can function to activate mitochondrial release of apoptotic factors [increased levels of UCP-2 have been shown to induce peroxide generation from mitochondria (28)], or whether free fatty acids (see above) are also required, needs to be determined. However, this pathway may be significant because it indicates a radiation-dependent transcriptional control of mitochondrial function (Fig. 4).

VDAC. VDAC-1 is part of a protein complex that includes the adenine nucleotide translocator (also induced in response to radiation) and cyclophilin D and is commonly referred to as the permeability transition (PT) pore. Opening of the PT pore, with release of apoptogenic factors, may be the ultimate checkpoint before commitment to apoptosis occurs, and it has been shown to be under the regulation of BCL-2 family members (29, 30). Indeed, Tsujimoto and co-workers (29) recently reported that both pro- and anti-apoptotic members of the BCL-2 family are capable of binding the PT pore though interactions with VDAC-1. In an elegant series of experiments, this group determined that BCL-2 is able to prevent VDAC-1 from opening liposomes, and that the pro-apoptotic family members reversed

this effect. In addition, cytochrome *c*, which otherwise would not fit through the channel formed by VDAC-1, was transported into VDAC-1 liposomes when recombinant BAX or BAK was added (29). While regulation of VDAC-1 at the site of the mitochondria was described in this report, no published study of which we are aware has shown a radiation-induced transcriptional response of VDAC-1 in cells about to undergo apoptosis.

VDAC-1 mRNA was strongly induced in the LYas cells, but not in LYar cells, in response to radiation exposure (Fig. 3*C*). While VDAC-1, VDAC-2, and VDAC-3 levels were somewhat elevated in untreated LYas cells, compared with LYar cells only VDAC-1 levels increased dramatically after irradiation.

Interestingly, it was previously reported that a mitochondrial translocase (hTOM 20, which is not present in our gene microarray) has a cytosolic domain that interacts directly with UCP, VDAC, hTOM 70, and BCL-2 (31). How all these players function in regulating apoptosis is yet to be determined; however, the regulation of expression of mRNA encoding mitochondrial proteins may be critical.

Conclusions

Determination of multigenic expression profiles with gene microarrays is rapidly becoming a valuable tool in deciphering complex regulatory pathways. A previous report, using serial analysis of gene expression, investigated p53-mediated apoptosis in a colon carcinoma cell line and revealed the induction of genes regulating oxidative stress, the generation of reactive oxygen species, and the initiation of mitochondrial damage (32). Our data support these findings in apoptosis-sensitive cells, by identifying two proteins that are involved in disrupting normal mitochondrial function (VDAC-1 and UCP-2), and they reveal in apoptosis-resistant cells a pathway that may prevent mitochondrial uncoupling by free fatty acids. We did not detect all the changes reported in the previous study, but this difference may be because of the use of ionizing radiation to induce p53 stabilization in the present experiments versus direct expression of adenovirus introduced, and perhaps overexpressed, p53 in the previous study, or to the different cell types used in the respective experiments (32).

Additionally, our data expand the knowledge of apoptosis susceptibility by defining the gene expression profile of closely related cells that do or do not undergo apoptosis. We previously reported that LYar cells have elevated BCL-2 levels that appear to increase intracellular GSH, primarily in the nucleus (5). Depleting intracellular GSH in the LYar cells reverses the ability of BCL-2 to block apoptosis, and decreases clonogenic survival, suggesting that the intracellular redox state of the cell, and not BCL-2 per se, is important for apoptosis resistance. Our current findings support this hypothesis, as the mRNAs for several proteins involved in increasing the antioxidant defense mechanisms of LYar cells are elevated. Interestingly, one of the genes that showed the greatest difference in expression between the LYar cells and the LYas cells was a member of a family of glutathione *S*-transferases (theta-like GST; GenBank no. U80819) previously identified and cloned by differential display in the LYar versus the LYas cells (2).

What causes this shift from sensitive to resistant phenotype? Our current model predicts that BCL-2 inhibits apoptosis through modulation of glutathione homeostasis and intracellular distribution, and that these alterations in turn change nuclear redox potential. Because many transcriptional enhancers bind DNA in a redox-sensitive mechanism, the changes in nuclear redox potential are expected to alter transcriptional responses affecting cellular phenotype (3, 5, 20, 21). The LYar cells arose spontaneously from an LYas clone in response to prolonged culture (4); therefore, in addition to BCL-2 overexpression, the expression of many other genes is also altered during the development of apoptosis resistance, as shown in this study. Our hypothesis that many of these transcriptional changes are caused by BCL-2-mediated nuclear GSH changes, can be tested by seeing whether altered gene expression occurs in LYas cells that have been infected with a retrovirus bringing in BCL-2.

We thank T. B. Breithaupt and R. E. Meyn for discussion and A. W. Tu for assistance with the manuscript preparation. This work was supported by a grant from the National Institutes of Health and a gift from the Phil N. Allen Fund. D.W.V. was supported in part by a National Institutes of Health Institutional Training Grant in Immunology (AI-0729015). D.L.H. was supported in part by a fellowship from the National Multiple Sclerosis Society.

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