Fas and Fas Ligand in Embryos and Adult Mice: Ligand Expression in Several Immune-privileged Tissues and Coexpression in Adult Tissues Characterized by Apoptotic Cell Turnover

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Abstract. The cell surface receptor Fas (FasR, Apo-1, CD95) and its ligand (FasL) are mediators of apoptosis that have been shown to be implicated in the peripheral deletion of autoimmune cells, activation-induced T cell death, and one of the two major cytolytic pathways mediated by $CD8^+$ cytolytic T cells. To gain further understanding of the Fas system, we have analyzed Fas and FasL expression during mouse development and in adult tissues. In developing mouse embryos, from 16.5 d onwards, Fas mRNA is detectable in distinct cell types of the developing sinus, thymus, lung, and liver, whereas FasL expression is restricted to submaxillary gland epithelial cells and the developing nervous system. Significant Fas and FasL expression were observed in several nonlymphoid cell types during embryogenesis, and generally Fas and FasL expression were not localized to characteristic sites of programmed cell death. In the adult mouse, RNase protection analysis revealed very wide expression of both Fas and FasL. Several tissues, including the thymus, lung, spleen, small intestine, large intestine, seminal vesicle, prostate, and uterus, clearly coexpress the two genes. Most tissues constitutively coexpressing Fas and FasL in the adult mouse are characterized by apoptotic cell turnover, and many of those expressing FasL are known to be immune privileged. It may be, therefore, that the Fas system is implicated in both the regulation of physiological cell turnover and the protection of particular tissues against potential lymphocyte-mediated damage.

APOPTOSIS is an active cell death process that takes place in a spectrum of physiological conditions such as embryonic development and normal cell turnover, but is also the mode of cell death resulting from $CD8^+$ T cell–mediated cytotoxicity (Steller, 1995). The mechanisms that regulate apoptotic cell death are therefore crucial for normal development and homeostasis.

The Fas receptor (FasR, Apo-1 CD95)[1] is a cysteine-rich type I membrane protein belonging to the tumor necrosis factor (TNF) NGF receptor family, and shows high sequence similarities to CD27, CD30, CD40, and the lymphotoxin-β receptor (Nagata and Golstein, 1995; Smith et al., 1994). Upon contact with cross-linking antibodies or the natural ligand (FasL), cells expressing Fas undergo apoptosis rapidly (Itoh et al., 1991; Trauth et al., 1989) by way of a rapid intracellular signaling pathway dependent on a distinct cytoplasmic motif called the "death domain" (Hofmann and Tschopp, 1995; Itoh and Nagata, 1993; Tartaglia et al., 1993). FasL is a type II membrane protein and a member of the protein superfamily consisting of TNF-α, lymphotoxin-α (TNF-β), lymphotoxin-β, CD27L, CD30L, CD40L, and 4-1BBL (Smith et al., 1994).

Considerable knowledge of the Fas system's biological relevance has been provided by mutant mice. Mice homozygous for *lpr* (lymphoproliferation) or *gld* (generalized disease) develop a progressive autoimmune disorder that resembles systemic lupus in humans (Cohen and Eisenberg, 1991). These mice also suffer from a large accumulation of nonmalignant $CD4^-CD8^-$ T cells in the spleen and lymph nodes. Genetic cloning and the analysis of gene structure has indicated that *lpr* is a recessive mutation in Fas (Watanabe et al., 1992*a*), whereas the *gld* phenotype is a mutation in the gene for FasL (Lynch et al., 1994; Takahashi et al., 1994).

The Fas system has been shown to play a crucial role in the peripheral deletion of autoimmune cells (Kotzin et al., 1988; Rozzo et al., 1994; Singer and Abbas, 1994) and in activation-induced T cell death (Alderson et al., 1995;

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1. *Abbreviations used in this paper*: FasL, Fas ligand; FasR, Apo-1 CD95, Fas receptor; nt, nucleotide; TNF, tumor necrosis factor; UTP, uridine 5′-triphosphate.

Brunner et al., 1995; Dhein et al., 1995). FasL is also an important effector molecule of cytolytic T lymphocytes, one of the two major cytolytic pathways of $CD8^+$ cytolytic T cells being dependent on Fas (Kagi et al., 1994; Lowin et al., 1994).

Although detailed studies have described the function of Fas and its ligand in the immune system, relatively little is known about its role outside of the lymphoid system. Recent studies have shown that both Fas and FasL are expressed in certain nonlymphoid tissues (Bellgrau et al., 1995; Griffith et al., 1995; Leithauser et al., 1993; Suda et al., 1993; Watanabe et al., 1992*b*), but little is known about their function(s) at these sites. To gain further understanding of the Fas system and an eventual role in mammalian development, we have analyzed Fas and FasL gene expression during mouse development and in adult tissues.

Materials and Methods

Materials. Adult NMRI female mice (Kleintierfarm, Madörin, Basel) were housed with males for one night and monitored for the appearance of a vaginal plug the next morning. Noontime on the day of the vaginal plug was considered as day 0.5 of gestation. Segments of uteri or, at later stages, whole embryos were dissected, embedded in Tissue-Tek (Miles, Ames Division, Elkhart, IN), frozen in precooled methylbutane, and stored at −70°C. For each time point, three to four embryos were analyzed, and 20–40 sections were prepared per embryo.

The polyclonal (PE62) and monoclonal (H11) antibodies used were directed against a peptide spanning amino acids 197–218 of mouse FasL (Hahne et al., 1995). The polyclonal rabbit antibody was affinity purified on its antigen. The monoclonal antineurofilament 200 antibody (Sigma Immunochemicals, St. Louis, MO) was a kind gift from Y. Sagot (University of Geneva, Geneva, Switzerland), and the monoclonal antivimentin antibody (MC7A3) was a kind gift from E. Reichmann (ISREC, Epalinges, Switzerland). Secondary antibodies were FITC-conjugated, affinity-purified $F(ab)_2$ fragment anti-rabbit or anti-rat IgG antibodies (Dianova, Hamburg, Germany or Sigma), biotinylated rabbit anti-mouse IgG antibodies (Dako, Glostrup, Denmark), and FITC sheep anti-mouse IgG/antibodies (Serotec, Oxford, UK).

Plasmid Constructions, In Vitro Transcription, RNA Isolation and RNase Protection Analysis. The plasmid containing the 1.5-kb mouse Fas cDNA was a kind gift from P. Erb (University of Basel, Basel, Switzerland), and was used as such for in vitro transcription of sense and antisense probes that were used for in situ hybridization. For RNase protection analysis, a HindIII/DraII fragment containing the 5′ 974-bp of this cDNA was subcloned into pBluescript KS (Stratagene, La Jolla, CA), and was subsequently linearized at the XhoI site to yield a protected fragment of ~203 nucleotides (nt). Mouse FasL cDNA was generated by PCR using primers 5′-ATG CAG CAG CCC GTG AAT TAC-3′ and 5′-CCA TAT CTG GCC AGT AGT GC-3′ designed to amplify cDNA corresponding to amino acid residues 1–237 of FasL, which was subsequently subcloned into a pBK-CMV vector (Stratagene). For RNase protection analysis, a PstI fragment containing the 3′ end of the FasL cDNA was deleted, leaving a 5′ 320-bp fragment of the cDNA in the pBK-CMV vector. This template was linearized at the EcoRI site to yield a protected fragment of ~320 nt. The pT7 RNA 18S plasmid (Ambion, Austin, TX), containing a highly conserved region of the human 18S ribosomal RNA gene, was linearized at the HindIII site to create a template that yields an 80-nt protected fragment that sometimes migrates as a doublet. Sense and antisense radiolabeled cRNAs were synthesized from the above templates by in vitro transcription with ^{32}P-labeled uridine 5′=triphosphate (UTP) (400 Ci/mmol), ^{35}S-labeled UTP (400 Ci/mmol), or ^{3}H-labeled UTP and CTP (40 and 20 Ci/mmol, respectively). For in situ hybridization, ^{35}S- and ^{3}H-labeled probes were subsequently reduced to an average size of 50–100 nt by mild alkaline hydrolysis as previously described (French et al., 1993). For the synthesis of ^{35}S-labeled probes, all reactions were performed in 10 mM DTT.

Total RNA was isolated from 20 selected adult tissues that were collected from 3-mo-old NMRI mice as described (French et al., 1993). For RNase protection assays, 15 μg of total RNA was hybridized for 16 h to ^{32}P-labeled Fas or FasL cRNAs (300,000 cpm/sample, specific activity 1.2×10^8 cpm/μg) along with a final twofold excess of cold plus ^{32}P-labeled 18S cRNAs (1,000 cpm/sample, specific activity 0.5×10^6 cpm/μg) at 42°C as described (Belin et al., 1989). A twofold excess of 18S cRNAs was previously shown to be sufficient to detect small variations in RNA loading. Unhybridized RNA was digested with RNase A, followed by proteinase K digestion, phenol/chloroform extraction, and sodium acetate precipitation. Hybridized RNA was then denatured at 90°C, separated in a 5% polyacrylamide gel, and exposed to x-ray films.

In Situ Hybridization and Immunofluorescence. Cryostat sections (6–8 μm) were air dried, fixed in 4% (wt/vol) paraformaldehyde in PBS, used immediately for immunofluorescence, or stored in 70% ethanol at 4°C before in situ hybridization. In situ hybridization and immunofluorescence were performed as described (French et al., 1992; 1993). After in situ hybridization, slides were either directly exposed to x-ray films (sections hybridized to ^{35}S-labeled cRNAs) and developed after 14 d of exposure, or immersed in NTB-2 emulsion (sections hybridized to ^{3}H-labeled cRNAs), developed after a 12–16-wk exposure, and counterstained in 1% methylene blue. Controls of specificity for *in situ* hybridizations included the systematic use of sense cRNA probes in each experiment. For immunofluorescence analysis, controls of specificity included incubation of sections with nonimmune rabbit or rat Igs, incubation in the absence of the first antibody, and in the case of FasL, preincubation of the primary antibody for 30 min with a 10-fold (wt/wt) excess of FasL (AA 197-218) or unspecific peptide.

Results

Mouse Embryos Contain Fas and FasL mRNA from 16.5 d Onwards

The regional distribution of Fas and FasL mRNA in mouse embryos was determined by in situ hybridization of the respective ^{35}S-labeled cRNA probes to sagittal sections of whole embryos ranging from 5.5 to 18.5 d of development, and subsequent exposure to x-ray films. Using this technique, Fas and FasL mRNA were detected in certain macroscopically identifiable organs from 16.5 d of development onwards (Fig. 1). No signal was detected with the sense Fas and FasL cRNA probes (Fig. 1 and not shown). As shown in Fig. 1, at 16.5 d of development, Fas mRNA is detectable in the developing sinus, thymus, lung, and liver. At the same stage of development, a different pattern of expression was observed for FasL, which is macroscopically detectable in the developing brain, spinal cord, and submaxillary gland. The regional distribution of Fas and FasL mRNA was very similar in 18.5-d-old embryos (not shown).

Fas mRNA Is Abundant in Developing Thymocytes and Upper Respiratory Tract Epithelial Cells

The cellular distribution of Fas mRNA in embryos ranging from 5.5 to 18.5 d of development was determined by in situ hybridization using ^{3}H-labeled cRNA probes. The strongest levels of embryonic Fas gene expression were detected in the thymus and upper respiratory tract. In the thymus, both the cortical and medullary regions were strongly labeled from 16.5 d of development onwards (Fig. 2, *A* and *B*), and the labeling observed is specific in that no signal was detectable in sections hybridized to the sense probe (Fig. 2, *C* and *D*). The distribution and morphology of the cells containing Fas transcripts suggest that thymocytes account for the majority of Fas gene expression at this stage of development. Considerable amounts of Fas mRNA were also detectable at 16.5 and 18.5 d of development in the respiratory epithelial cells of the developing nasal cavities and sinuses (Fig. 2, *E–H*). A clear cutoff was seen at the limit between the respiratory and the olfactory epithelium, with no Fas mRNA detectable in the latter

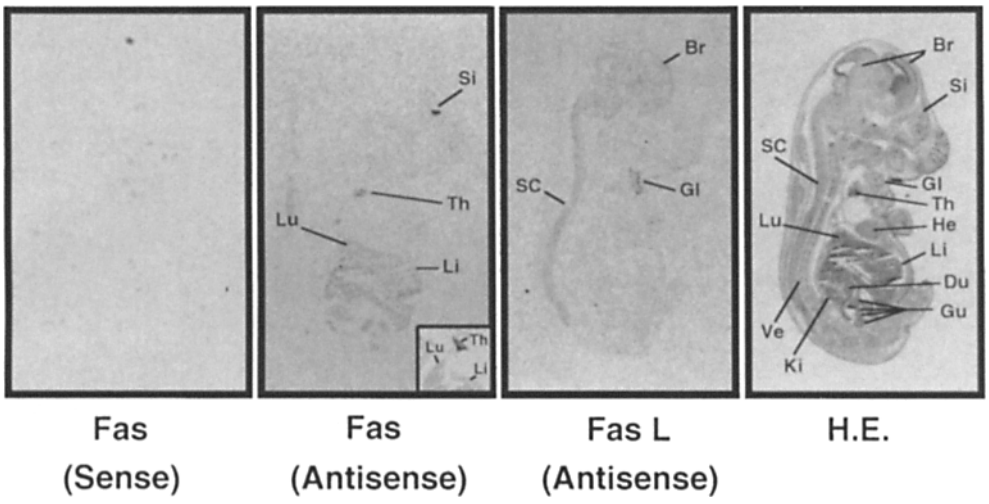

Figure 1. Regional distribution of Fas and FasL mRNA in 16.5-d-old mouse embryos as assessed by in situ hybridization of ^{35}S-labeled cRNA probes to sagittal cryostat tissue sections. Fas mRNA (*Fas*) is detectable in the developing sinus (*Si*), thymus (*Th*), lung (*Lu*), and liver (*Li*). The inset shows the thymus, lung, and liver several sections further in an embryo of the same age. FasL mRNA (*FasL*) is detectable in the developing brain (*Br*), spinal cord (*SC*), and submaxillary gland (*Gl*). No signal is detected in an adjacent section hybridized to the Fas sense probe (*Sense*). The section on the far right was counterstained with hematoxylin and eosin (*H.E.*), and it shows the localization of macroscopically identifiable organs. Vertebrae (*Ve*), kidney (*Ki*), gut (*Gu*), duodenum (*Du*), and heart (*He*). Photographs were taken after 15 d (*Fas*, *FasL*, and *Sense*) of exposure at room temperature.

(Fig. 2, *E* and *F*). Less intense, yet still significant Fas expression was seen in the liver from 16.5 d onwards (Fig. 2, *I* and *J*). The developing liver is a predominant site of fetal hematopoiesis during embryogenesis, and in situ hybridization clearly shows that Fas mRNA is restricted to cells morphologically identified as hepatocytes (Fig. 2, *I* and *J*, *arrows*), whereas no message is detectable in the characteristically more darkly counterstained groups of fetal hematopoietic cells. Lower but significant levels of Fas mRNA were also detectable in epithelial cells of the developing lung and gut from 16.5 d of development onwards (Table I). In the lung, at 16.5 d of development, Fas mRNA was localized to epithelial cells of developing bronchioles, whereas in 18.5-d-old embryos, message was also detectable in developing alveoli (Table I). Taken together, Fas gene expression does not appear to be exclusively restricted to cells of the lymphoid cell lineage during embryogenesis, but is also detectable in hepatocytes and certain epithelial cell types.

Embryonic FasL Gene Expression Is Detected in Neurons and Submaxillary Gland Epithelial Cells. In situ hybridization of the FasL cRNA to cryostat sections of developing mouse embryos also revealed specific labeling in distinct cell types from 16.5 d of development onwards (Table I). For example, high levels of FasL mRNA were detectable within certain acinar glandular cells of the developing submaxillary gland (Fig. 3, *A* and *B*). In contrast, no signal was detectable in the epithelium lining the ducts of the submaxillary gland (Fig. 3, *A* and *B*) or in sections hybridized to the sense probe (Fig. 3, *C* and *D*). Analysis by immunofluorescence using an affinity-purified antibody directed against a peptide of mouse FasL (Hahne et al., 1995) revealed an identical pattern of staining within the salivary gland, with strong labeling present on certain glandular epithelial cells (Fig. 3, *E* and *F*). Specificity of this immunolabeling for FasL was confirmed by the identical staining observed using a mAb directed against the same domain of FasL, the abrogation of staining by coincubation of the antibody with an excess of FasL peptide (Fig. 3 *G*), and the absence of staining with control Igs or in the absence of the first antibody. The developing nervous system (brain and spinal cord) was shown to contain significant levels of FasL mRNA from 16.5 d onwards by in situ hybridization (Fig. 1 and Table I). Analysis by immunofluorescence with anti-FasL antibodies revealed clear and specific labeling of neurons and axons throughout the nervous system. Strong FasL expression in neurons and axons of the developing spinal ganglia is illustrated in Fig. 3, *H* and *I*. Similar FasL expression was detectable by immunofluorescence in axons below the sensory epithelium of the developing inner ear (Fig. 3 *J*). Identification of this structure was confirmed by an identical staining pattern observed on adjacent sections with an antineurofilament antibody (Fig. 3 *K*). Taken together, from 16.5 d of embryogenesis onwards, FasL gene expression is not restricted to the developing lymphoid system, and it predominates in nonlymphoid cell types within the developing nervous system and submaxillary gland.

Coexpression of Fas and FasL Genes in Several Adult Mouse Tissues. Fas and FasL mRNA content was assessed in 20 adult mouse tissues by RNase protection analysis. As shown in Fig. 4, both Fas and FasL are widely expressed in adult mouse tissues, and Fas appears on average more abundant as revealed by the long exposure time needed to detected FasL mRNA.

Fas mRNA was detected in all tissues tested (Fig. 4 *A*). High amounts of Fas mRNA were detectable in the skin, heart, lung, thymus, small intestine, and liver, and moderate amounts were detectable in the muscle, bone, spleen, stomach, large intestine, adrenal gland, seminal vesicle, prostate, and uterus. A protected mRNA fragment is also clearly detectable, although in lower amounts, in the brain, kidney, pancreas, testis, and ovary.

Using the same assay, we also observed wide expression of the FasL gene, although at lower levels (Fig. 4 *B*). Highest levels of FasL message were detectable in the spleen, small intestine, testis, and uterus; comparatively moderate levels in the lung, thymus, large intestine, and seminal ves-

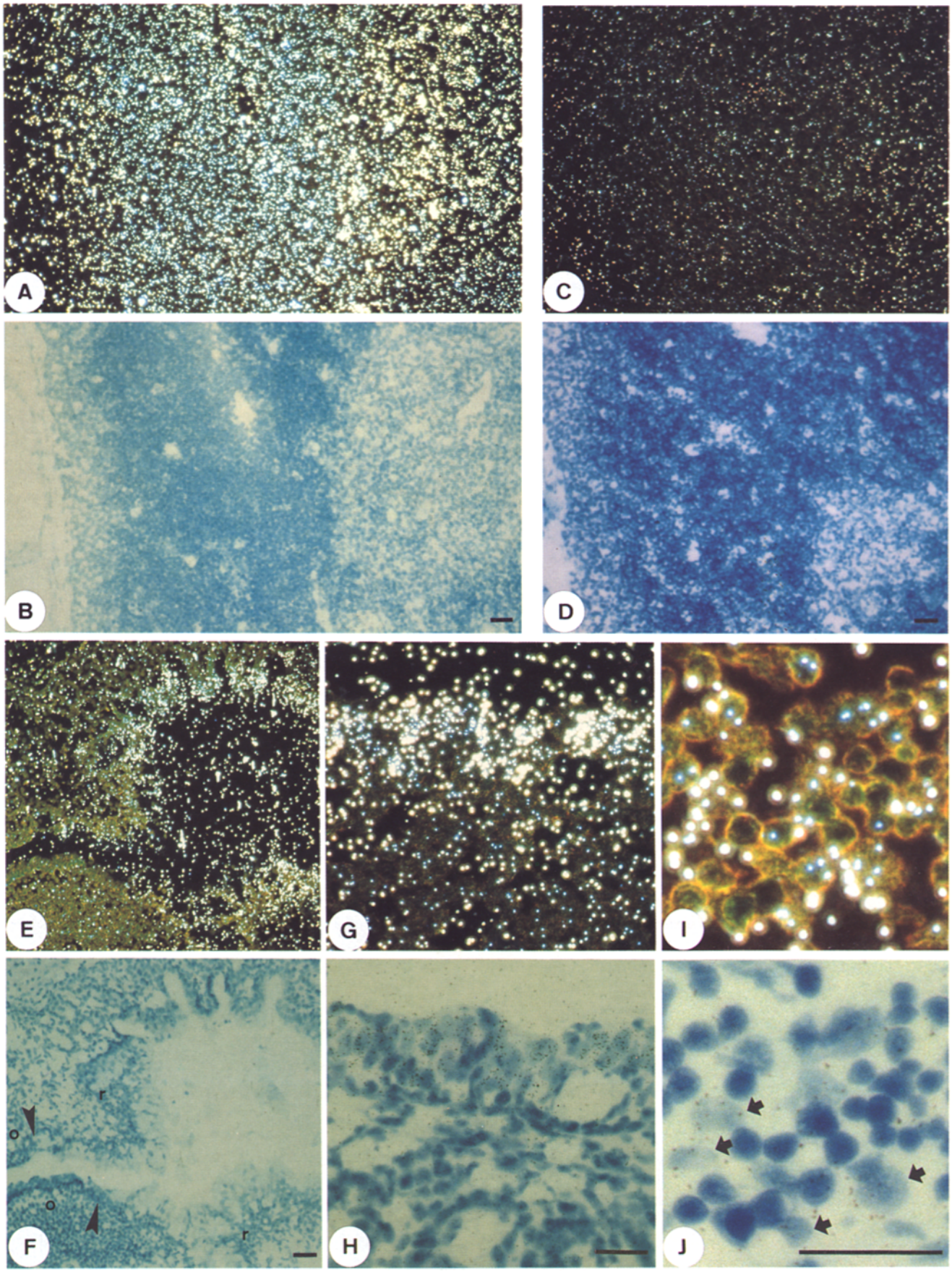
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Table I. Localization of Fas and FasL mRNA in 16.5- and 18.5-d-old Mouse Embryos

	16.5 d		18.5 d	
	Fas	FasL	Fas	FasL
Epithelia				
Keratinocytes	−	−	−	−
Choroid plexus	−	−	−	−
Respiratory epithelium	+++	−	+++	+
Otic epithelium	−	−	−	−
Retinal pigment epithelium	−	−	−	−
Proliferative lens epithelium	−	−	−	−
Salivary glands	−	+++	−	+++
Bronchial epithelium	+	−	++	−
Stomach	−	−	−	−
Intestine	+	−	+	−
Kidney tubular epithelium	−	−	−	−
Testes	NS	NS	NS	NS
Biliary epithelium	−	−	−	−
Pancreatic acini and ductules	−	−	−	−
Hepatocytes	+	−	++	−
Ventricular neuroepithelium	−	−	−	−
Thymus	++	+	++	+
Spleen	−	−	−	−
Adrenal gland	−	−	−	−
Hematopoietic tissue	−	−	−	−
Neuronal tissue				
Brain	−	++	−	++
Spinal cord	−	++	−	++
Photoreceptor layers of the eye	−	−	−	−
Bone (osteoclasts or osteoblasts)	−	−	−	−
Endothelia	−	−	−	−
Mesenchyme				
Kidney	−	−	−	−
Lung	−	−	−	−
Skeletal muscle	−	−	−	−
Heart muscle (ventricle)	−	−	−	−

Gene expression was asssessed in a variety of tissues semiquantitatively by in situ hybridization of ^{3}H-labeled murine Fas or FasL cRNA probes to frozen cryostat sections of 16.5- and 18.5-d-old embryos. −, no mRNA detectable; +, weak labeling; ++, moderate labeling; +++, strong labeling; *NS*, not studied. No Fas or FasL mRNA was detected in 5.5-, 6.5-, 7.5-, 9.5-, 10.5-, 12.5-, and 14.5-d-old embryos (not shown).

icle; and low levels in the skin, muscle, bone, brain, kidney, stomach, adrenal gland, prostate, and ovary. In the heart, liver, and pancreas, no protected FasL mRNA fragment was detectable after 16 d of exposure.

In the above assays, no protected fragments were observed with either the Fas or FasL probes in tRNA controls, and equal loading of total RNAs was confirmed by simultaneously hybridizing all samples to an 18S rRNA probe as an internal control. In the FasL RNAse protection assay, a band corresponding to residual full-length undigested FasL probe can be seen in certain samples.

Despite the overall difference in the levels of Fas and FasL gene expression in adult tissues, RNase protection analysis reproducibly demonstrated that both genes are widely expressed and that several adult mouse tissues, including the thymus, lung, spleen, small intestine, large intestine, seminal vesicle, prostate, and uterus, coexpress Fas and FasL.

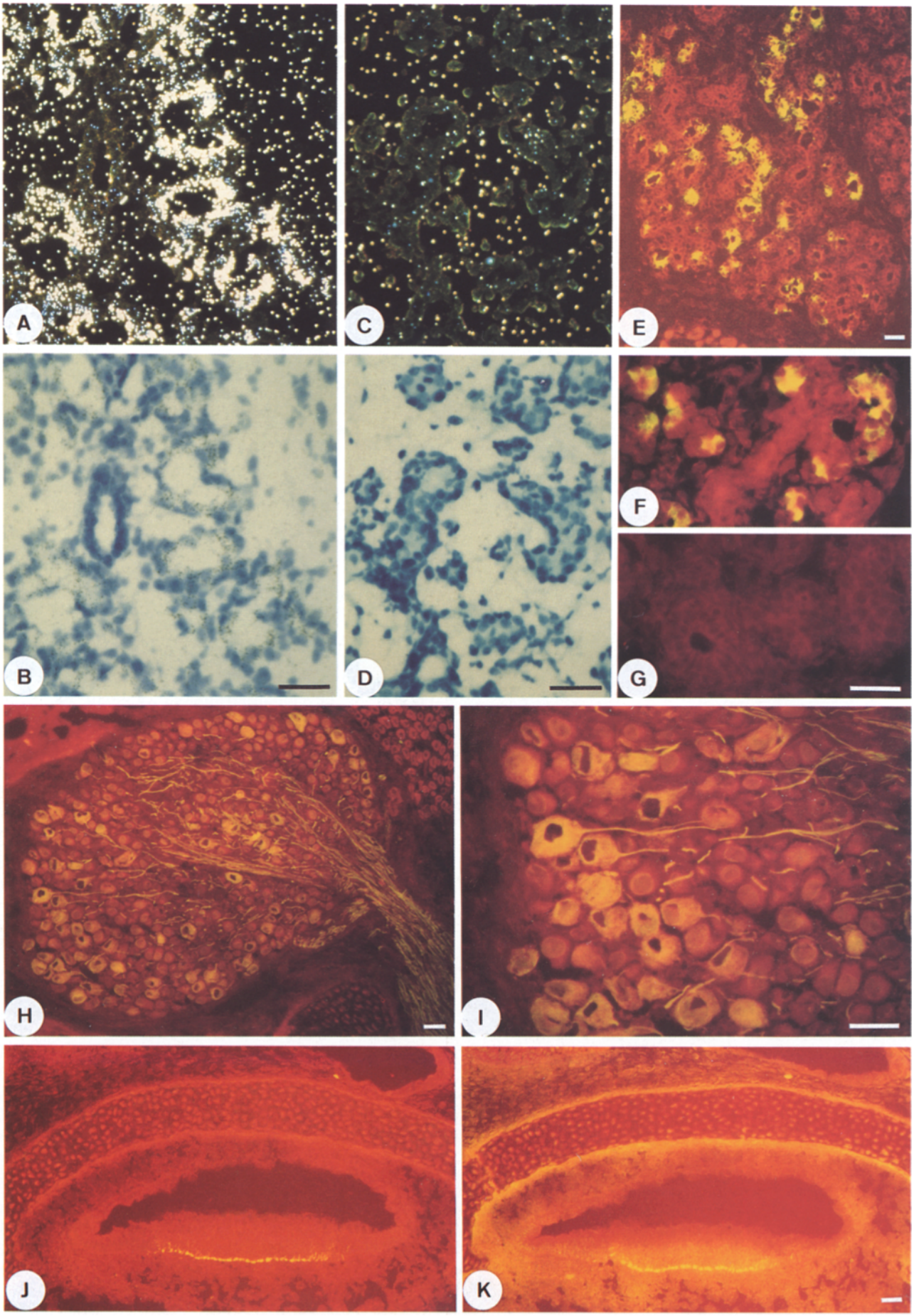
Sertoli Cells Account for the Strong FasL Gene Expression Observed in the Adult Testis. To determine whether the FasL gene expression observed by RNase protection analysis in adult tissues may result from cell types other than passenger leukocytes, we have analyzed FasL expression in the adult testis, where lymphocytes are rare and FasL mRNA is abundant. Analysis by immunofluorescence revealed a distinct pattern of labeling in the cells lining seminiferous tubules that is reminiscent of Sertoli cells (Fig. 5 *A*). Histological features of these cells, as observed on adjacent sections stained with hematoxylin and eosin, (not shown) and labeling of adjacent sections with antibodies to vimentin (Fig. 5 *C*), an intermediate filament that is specific for Sertoli cells within seminiferous tubules (Stathopoulos et al., 1989), strongly suggest that FasL is expressed by Sertoli cells in the testis. Recent evidence that supports this result has been provided by reverse transcription PCR analysis of FasL gene expression in primary Sertoli cell cultures (Bellgrau et al., 1995). Therefore, although FasL is known to be strongly expressed by lymphoid cells in the adult mouse, the above experiments clearly demonstrate that certain nonlymphoid cells also express FasL strongly.

Discussion

In this study, we analyzed the expression of Fas and FasL in mouse embryos and in the adult mouse. Our results show that Fas and FasL expression are restricted to distinct lymphoid and nonlymphoid tissues during late embryogenesis of the mouse embryo, and clearly are not only expressed by developing lymphoid cells. In the adult mouse, Fas and FasL are widely expressed, and interestingly, several tissues including the thymus, lung, spleen, small intestine, large intestine, seminal vesicle, prostate, and uterus appear to coexpress the two genes. In the adult, similarly to the embryo, FasL gene expression does not appear to be strictly restricted to lymphoid cell types, since significant gene expression is observed in several nonlymphoid tissues. The demonstration of strong FasL expression by Sertoli cells in the testis suggests furthermore that passenger lymphocytes cannot account for the FasL expression that is detected in all adult nonlymphoid tissues.

Apoptosis is one of the cellular processes that are essential for the proper development and shaping of tissues during development. For example, during embryogenesis, apoptotic cell death takes place in the regressing interdigital tissue of developing limb buds, in epithelial cells lining the palatal shelves before their fusion, in the duodenal mucosa during formation of the villi, and while connections are made in the nervous system (Wyllie et al., 1980). Analysis of Fas and FasL expression in mouse embryos from 5.5 d of development onwards did not reveal expression at

Figure 2. Microscopical localization of Fas mRNA in embryonic mouse tissues by in situ hybridization using ^{3}H-labeled murine Fas cRNAs. Fas mRNA is detected in the thymus (*A* and *B*), respiratory epithelium lining nasal cavities (*E–H*), and hepatocytes (*arrows*) of the liver (*I* and *J*) of 16.5-d-old embryos. Note the sharp transition (*arrowheads*) in expression between the respiratory (*r*) and olfactive (*o*) epithelia (*E* and *F*). *C* and *D* show the thymus of a 16.5-d-old embryo hybridized to the sense Fas cRNA probe. *A*, *C*, *E*, *G*, and *I* are dark-field micrographs; *B*, *D*, *F*, *H*, and *J* are the corresponding light-field micrographs. Bars, 33 μm.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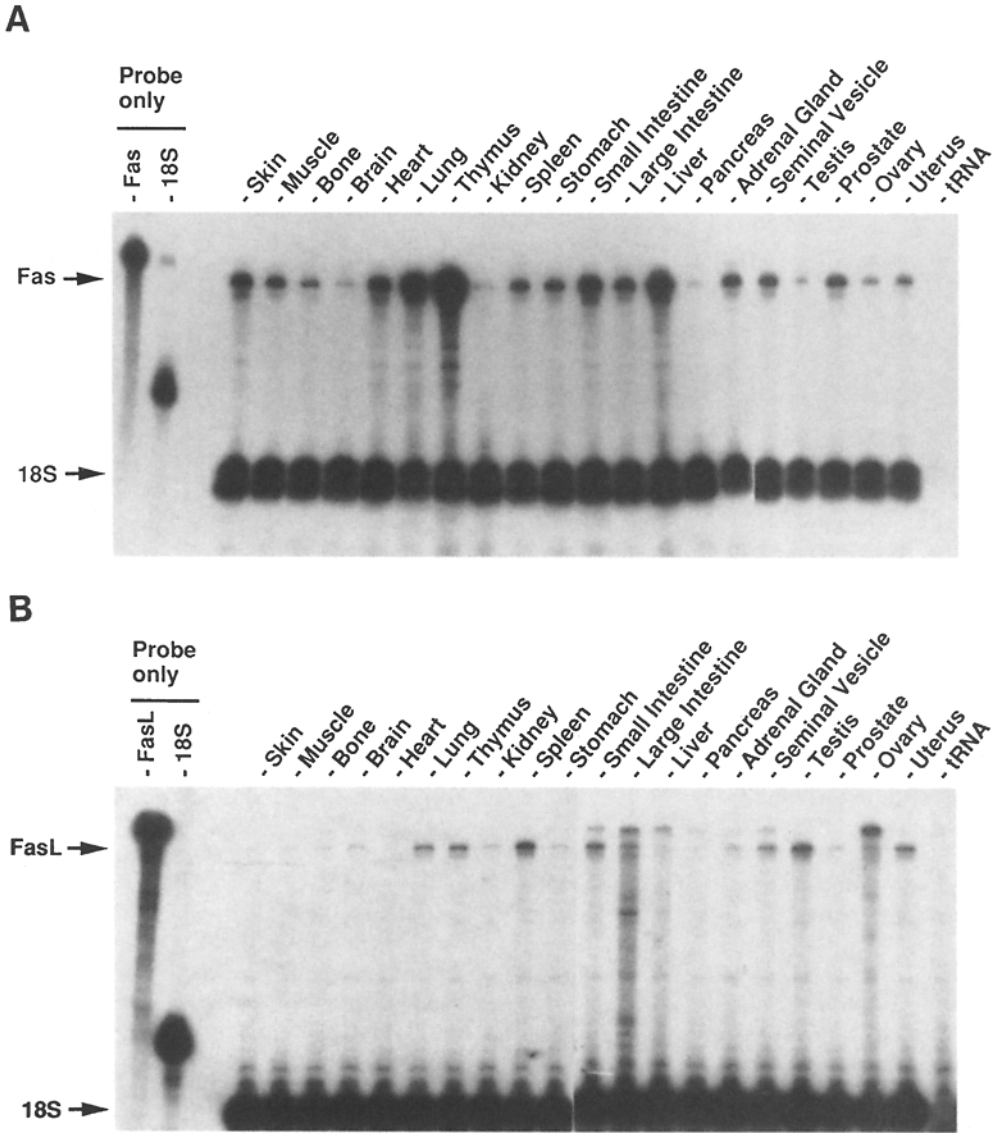

Figure 4. RNase protection analysis of Fas (*A*) and FasL (*B*) mRNA in adult mouse tissues. 15 μg of total RNA prepared from the indicated tissues were hybridized to the Fas or FasL riboprobes along with an 18S rRNA probe as an internal control. Protected fragments corresponding to Fas, FasL, and 18S are indicated by arrows. In *A* and *B*, riboprobes were also hybridized to tRNA as a negative control. A band corresponding to residual full-length undigested FasL probe can be seen in certain lanes (small intestine to seminal vesicle and ovary in *B*). Exposure time at −80°C was 4 d for *A* and 16 d for *B*.

known sites of apoptotic cell death during embyogenesis, except in the developing nervous system. At 16.5 and 18.5 d of development, FasL expression was detectable in neurons and axons of the brain, the spinal cord, and spinal ganglia. In vertebrates, during the period when neurons establish synaptic connections, 20–80% of all neurons in the central and peripheral nervous systems die (Oppenheim, 1991). In the chick, the majority of neuronal cell death takes place between the 13th and 17th day of development (Oppenheim, 1991). Although the time of developmental neuronal death in the mouse has not been well characterized, the site and period of FasL expression coincides approximately with the extrapolated period of ongoing cell death in the mouse nervous system. Coexpression and colocalization of Fas was not detected in the developing nervous system. Although we cannot exclude the presence of low amounts of Fas undetectable by in situ hybridization, the relevance of FasL expression in the developing nervous system in the absence of detectable Fas and its possible implication in developmental neuronal cell death remains to be determined. Since no abnormalities in embryonic development of l*pr* and *gld* mice have been described to date, it may be that FasL expression is not implicated in neuronal death, but rather in the protection of the

Figure 3. Microscopical localization of FasL mRNA and protein in embryonic mouse tissues by in situ hybridization using ^{3}H-labeled murine FasL cRNAs and immunofluorescence using a polyclonal anti-FasL antibody. FasL mRNA (*A* and *B*) and protein (*E* and *F*) are detected in the acinar glandular cells of the submaxillary gland of 16.5-d-old embryos. *C* and *D* are sections adjacent to *A* and *B* hybridized to sense FasL cRNAs. *G* is a section adjacent to *F* in which the anti-FasL Ab was preincubated with an excess of FasL peptide. FasL is also clearly detectable in neuronal bodies and axons of spinal ganglia in 16.5-d-old embryos (*H* and *I*). In the developing inner ear of 16.5-d-old embryos, FasL is localized to a distinct neuronal structure beneath the sensory epithelium (*J*), which is also labeled by an antineurofilament antibody (*K*). *A* is a dark-field micrograph, and *B* is the corresponding light-field micrograph. Bars, 33 μm.

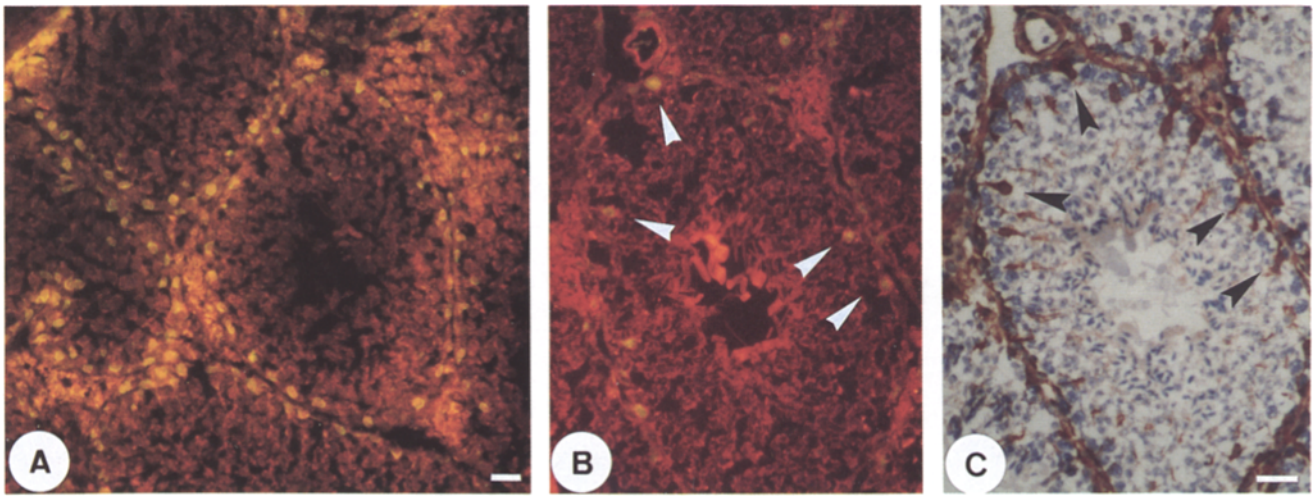

Figure 5. Microscopical localization of FasL in the adult mouse testis by immunofluorescence using polyclonal anti-FasL antibody. FasL (*A*) is detected in distinct cells located at the base of seminiferous tubules. Staining of adjacent sections with FasL (*B*) and vimentin (*C*) antibodies suggests that vimentin-positive Sertoli cells express FasL (*arrows*). Bars, 33 μm.

nervous system. Indeed, besides the blood–brain barrier, FasL expression on cells of the central nervous system may comprise a second protection mechanism necessary for preventing lymphocyte-mediated damage within the central nervous system. The brain is known to be an immune-privileged site, and similarly to the adult eye and testis (Bellgrau et al., 1995; Griffith et al., 1995), FasL expression in the embryonic and adult brain may protect the brain from potentially dangerous immune reactions.

In contrast to the restricted expression of Fas and FasL in embryos, expression of these genes is much more widespread in adult mice. Previous Northern blot analyses of a limited number of adult mouse tissues has shown Fas gene expression in the lung, heart, liver, thymus, kidney and ovary (Watanabe et al., 1992*b*), whereas FasL has to date been detected in the testis, small intestine, kidney, lung, spleen, thymus, and eye (Griffith et al., 1995; Suda et al., 1993). The analyses performed in this study on a larger array of mouse tissues using a more sensitive technique revealed wider Fas and FasL gene expression than has been so far shown in previous studies. Although Fas and FasL mRNA levels clearly differ within the same tissues, our results show that several tissues, including the thymus, lung, spleen, small intestine, large intestine, seminal vesicle, prostate, and uterus, clearly coexpress the two genes. Fas and FasL gene expression in these tissues may result from passenger leukocytes (Suda et al., 1995). Previous reports, however, have shown that Fas is expressed by cells other than leukocytes (Leithauser et al., 1993), and the present report demonstrates that this is also the case for FasL, which is strongly expressed by developing neurons, submaxillary gland epithelial cells, and Sertoli cells within the adult testis. Taken together, it clearly appears that expression of Fas and FasL is not solely restricted to lymphoid cells. It may be, therefore, that the Fas system is also associated with apoptosis of certain nonlymphoid cell types other than those known to date (Ogasawara et al., 1993). Interestingly, several of the tissues that coexpress Fas and FasL are also characterized by high rates of cell turnover. Furthermore, in the thymus, spleen, small intestine, prostate, and uterus, apoptotic cell death has been identified, and moreover, evidence suggests that in several of these tissues apoptosis is implicated in the control of cell turnover and tissue homeostasis (Benedetti et al., 1988; Gavrieli et al., 1992; Kerr and Searle, 1973; Liu et al., 1989; Rotello et al., 1992; Surh and Sprent, 1994). Coexpression of Fas and FasL in these tissues therefore raises the possibility that the Fas system may be implicated in apoptotic cell death during physiological cell turnover. Evidence in support of this hypothesis is provided by *lpr* (lymphoproliferation) and *gld* (generalized disease) mice, which suffer from a large accumulation of nonmalignant $CD4^-CD8^-$ T cells in the spleen and lymph nodes caused by defective Fas-mediated apoptosis.

Alternatively, FasL expression in nonlymphoid tissues may be implicated in providing protection from immune damage caused by Fas-bearing T cells. It has recently been shown that FasL expression in the testis and eye confers an immune-privileged status to these tissues, thereby enabling cell types expressing FasL to delete by apoptosis Fas-bearing T cells that enter the tissue (Bellgrau et al., 1995; Griffith et al., 1995). Several tissues other than the testis and eye are known to be immune-privileged (Streilein, 1995). Interestingly, several of these tissues, including the brain, testis, adrenal gland, uterus, ovary, and prostate, were shown by RNase protection analysis to express the FasL gene. It therefore appears likely from this study, that in addition to the eye and testis, constitutive FasL expression may also be implicated in the maintenance of an immune privilege within the brain, adrenal gland, uterus, ovary, and prostate. Further investigations will be required to confirm this hypothesis.

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