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Detection of Immunoglobulin Light-chain mRNA in Lymphoid Tissues Using a Practical *In Situ* Hybridization Method

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The identification of immunoglobulin protein in routinely fixed and paraffin-embedded sections using antibodies combined with immunoperoxidase or similar techniques of detection is often problematic. We developed an* in situ *hybridization methodology for the identification of light-chain mRNA that is applicable to formalin-fixed, paraffin-embedded tissues, using either radiolabeled or biotinylated oligonucleotide probes based on the kappa and lambda light-chain gene-constant regions. Reactive plasma cells can be consistently identified in reactive lymphoid tissues, and a monotypic pattern of light-chain mRNA restriction was seen in each of eight cases of multiple myeloma/plasmacytoma. Immunoblasts and germinal center cells also are labeled in reactive lymphoid tissues. Using 35S-labeled probes, 29 of 93 cases (30%) of non-Hodgkin's lymphomas had detectable light-chain mRNA, while 19% of non-Hodgkin's lymphomas were positive using biotinylated probes. (Am J Pathol 1990, 137:979–988)

The identification of intracellular immunoglobulin protein using antibodies combined with immunoperoxidase or similar techniques is recognized as a routine method to identify normal plasma cells.[1] In addition, the technique may be useful in cases of plasmacytoma and multiple myeloma, both through the identification of their plasmacytic character as well as through the establishment of monoclonality with the demonstration of light-chain restriction to either kappa or lambda light chain.[2,3] However, in a practical setting, the demonstration of light chains in plasma cells and tumors is often problematic. In paraffin sections, there is frequently a lack of staining for either kappa or lambda light chain, or preferential expression of one light chain with a significant population of cells that is unstained for either light chain. In addition, there is often uptake of polytypic immunoglobulin by necrotic tissue, and confounding extracellular immunoglobulin protein. Thus in one large hematopathology service, 46% of the studies of immunoglobulin expression in paraffin sections performed during a 1-year period were not helpful due to one or more factors.[4] The identification of immunoglobulin protein in the cytoplasm or on the membrane of B lymphocytes and their tumors that have been routinely fixed and paraffin embedded using antibodies is even more difficult, and, with the exception of one study,[5] successful in only a few cases.[6,7]

In the past several years, *in situ* hybridization has been used as a technique to identify and localize specific messenger RNAs (mRNAs), including immunoglobulin light-chain mRNAs, within tissue sections.[8–14] Although many initial protocols often were unreliable and lacked a high degree of sensitivity, marked improvements have been made in the technique, and it is now potentially within the realm of the diagnostic pathologist's use. The most sensitive protocols still use a radioisotope, such as 35S, combined with autoradiography for detection; however excellent results may be obtained using biotinylated probes combined with an avidin-alkaline phosphatase or similar detection system.[10–14]

In the current report, the authors describe an *in situ* hybridization procedure using oligonucleotide probes for the detection of kappa and lambda light-chain mRNA in paraffin-embedded tissue sections. The distribution of light-chain mRNA in normal lymphoid tissues is described, and preliminary studies on its expression in paraffin-embedded tissue sections from cases of plasma cell neoplasms and malignant lymphoma are reported.

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Table 1. *Nucleotide Sequence of Oligonucleotide Probes Complementary to Human Kappa and Lambda Constant-region mRNA*

Kappa 5′		3′
10	20	30
GATGAAGACA	GATGGTGCAG	CCACAGTTCC
CAGCAGGCAC	ACAACAGAGG	CAGTTCCAGA*
GGGAGTTACC	CGATTGGAGG	GCGTTATCCA
AGACTTTGTG	TTTCTCGTAG	TCTGCTTTGC
Lambda 5′		**3′**
10	20	30
TTCCAGGCCA	CTGTCACAGC	TCCCGGGTAG
AGCTTCTGTG	GGACTTCCAC	TGCTCGGGCG
TGTAGGGGCC	ACTGTCTTCT	CCACGGTGCT*
GTCACTGATC	AGACACACTA	GTGTGGCCTT
AACAGAGTGA	CAGTGGGGTT	GGCCTTGGGC
TGTTGTTGCT	CTGTTTGGAG	GGTTTGGTGG

* Probes used in 35S-labeling studies.

Methods

Cases and Preparation of Tissues

Paraffin-embedded tissues from cases of hyperplastic tonsils, hyperplastic lymph nodes, plasmacytoma/multiple myeloma, small lymphocytic lymphoma, follicular lymphoma, B-lineage diffuse large cell lymphoma, and Hodgkin's disease were studied. Most cases were fixed in neutral buffered formalin in a routine manner and embedded in paraffin. A few cases were fixed in B5. The cases of neoplasms had been previously characterized for immunoglobulin protein expression by routine immunohistologic methods, performed on paraffin sections in the cases of plasma cell neoplasms and on frozen sections in the cases of malignant lymphomas.[15] For the current study, 5-μ paraffin-embedded tissue sections were cut using gloves and a gelatin-free water bath onto glass slides that had been pretreated with 3-aminopropyltriethoxysilane (Sigma Chemical Co., St. Louis, MO), baked for 45 minutes at 65°C, and stored at room temperature until use. Several cases also were snap frozen, embedded in OCT, and 5-μ tissue sections were cut using gloves onto slides that had been pretreated with 3-aminopropyltriethoxysilane. The slides were air dried, fixed in freshly prepared 4% paraformaldehyde for 10 minutes, washed in phosphate-buffered saline, dehydrated, and stored at −20°C until use.

Probes and Labeling

Thirteen 30-base oligodeoxyribonucleotides were used. Four were complementary to human kappa chain mRNA and six were complementary to human lambda light-chain mRNA; each was prepared from the published constant region coding sequence for each gene (Table 1).[16,17] Two were the same sense as nucleic acid sequences of mRNA for each corresponding gene and were used as negative controls. One was a poly T sequence used to assess the mRNA preservation in the paraffin-embedded tissues. The probes were synthesized using the solid-phase phosphoramidite procedure with an Applied Biosystems DNA synthesizer (Foster City, CA) and purified by polyacrylamide gel electrophoresis or purchased in a purified form from Operon Technologies, Inc. (San Pablo, CA).

The probes were labeled with 35S or biotin. Probes were labeled at their 3′ end as follows: either 0.5 μg of probe was labeled with 5 μl of biotin-11-dUTP (Sigma) or 5 μl of 35S-dCTP (1200 Ci/mmol, New England Nuclear; Boston, MA) with 5 μl of terminal deoxynucleotidyl transferase (TdT) (Bethesda Research Laboratories, Gaithersburg, MD) and 10 μl of 5× TdT buffer (100 mmol/l [millimolar] cacodylate, 1 mmol/l $MnCl_2$, 1 mmol/l $MgCl_2$, 0.1 mmol/l dithiothreitol) in a total volume of 50 μl for 5 minutes, or 3.5 μg of probe was labeled with 16.7 μl of biotin-11-dUTP or 16.7 μl of 35S-dCTP with 1.5 μl of TdT and 10 μl of 5× TdT buffer in a total volume of 50 μl for 4 hours. The reaction was stopped by immersion in an ice bath. These reaction conditions were expected to add approximately three to four labeled nucleotides to the 3′ end. Some of the probes also were labeled at their 5′ end with one biotin molecule through an amino-alkylphosphoramide linker arm. The probes were extracted in a 50-μl mixture of phenol:chloroform:isoamyl alcohol (50:49:1), vortexed, 1 μl of 25 mg/ml tRNA added, revortexed, and then spun down in a centrifuge for 3 to 4 minutes. The upper (aqueous) phase was removed and the probe was precipitated with a 1:10 volume of 3 mol/l (molar) sodium acetate and 2× volume ethanol, centrifuged, washed with ethanol, dried, and resuspended in water treated with diethylpyrocarbonate (Sigma) to a concentration of 10 ng/μl. All probes were tested on positive control tissues before use, as a check on the labeling procedure. The specific activity of the 35S-labeled probes was approximately 2×10^7 cpm/μg.

Hybridization and Wash Procedures

All solutions used were prepared with water treated with diethylpyrocarbonate. Paraffin sections were deparaffinized in xylene, dehydrated in ethanol, and air-dried. The sections were digested for 2 to 10 minutes with pronase (nuclease free; Cal Biochem, La Jolla, CA) at a concentration of 1 mg/ml in 50 mmol/l TRIS-hydrochloride pH 7.5, 5 mmol/l EDTA; washed in 2 mg/ml glycine in 0.1 TRIS-hydrochloride pH 7.5, 0.1 mol/l sodium chloride, and then dehydrated through a graded alcohol series and air dried. In selected negative control studies, a ribonuclease step

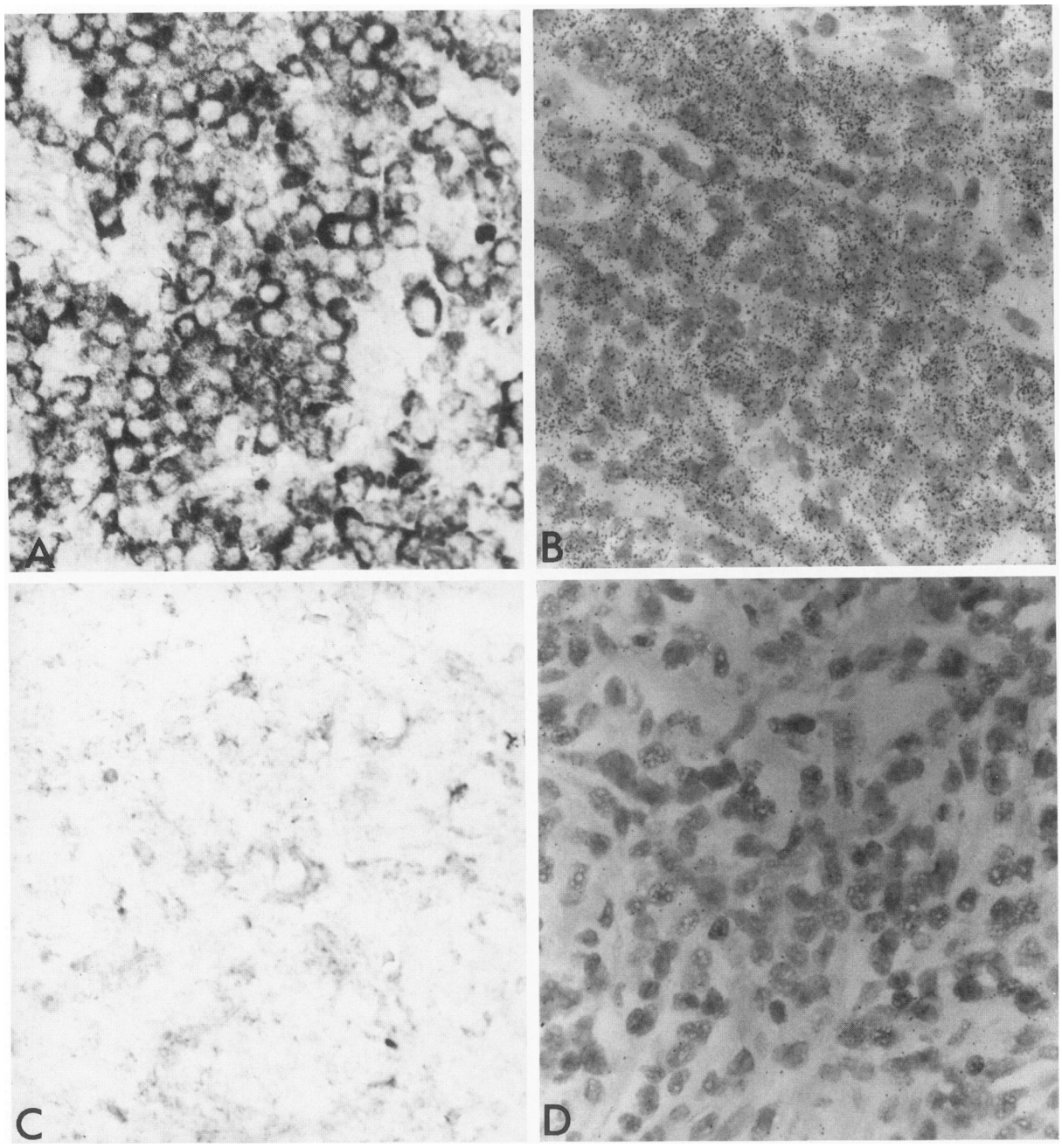

Figure 1. *Kappa protein-expressing case of multiple myeloma, paraffin sections.* A, *shows results of* in situ *hybridization with a cocktail of biotinylated oligonucleotide probes for kappa mRNA.* C *shows results using a cocktail of biotinylated oligonucleotide probes for lambda mRNA. In* B *and* D *are similar studies using 35S-radiolabeled probes and autoradiography (×400).*

was then performed with 0.2 U/μl ribonuclease T1 (Sigma) at 37°C for 60 minutes using buffer conditions recommended by the manufacturer. Then the sections were prehybridized with a solution containing 20 mmol/l sodium phosphate (pH 7.4), 1× Denhardt's solution (0.02% [w/v] polyvinyl pyrrolidone-40, 0.02% [w/v] Ficoll 400, 0.02% [w/v]〉 bovine serum albumin fraction V), and 10% (w/v) dextran sulfate for 2 hours at 37°C, and then hybridized for 2 hours to overnight with a solution containing 50% (v/v) formamide, 10% (w/v) dextran sulfate, 20 mmol/l sodium phosphate (pH 7.4), 3× SSC (0.45 mol/l sodium chloride, 0.045 sodium citrate), 1× Denhardt's solution, 100 μg/ml sheared, denatured salmon sperm, and 125 μg/ml yeast tRNA along with the appropriate amount of probe. Probes were used at a concentration of 0.5 ng/μl with 2-hour hybridizations and at 0.25 ng/μl with overnight hybridizations. When 35S-labeled probes were used, 2 mmol/l dithiothreitol also was added to the hybrid-

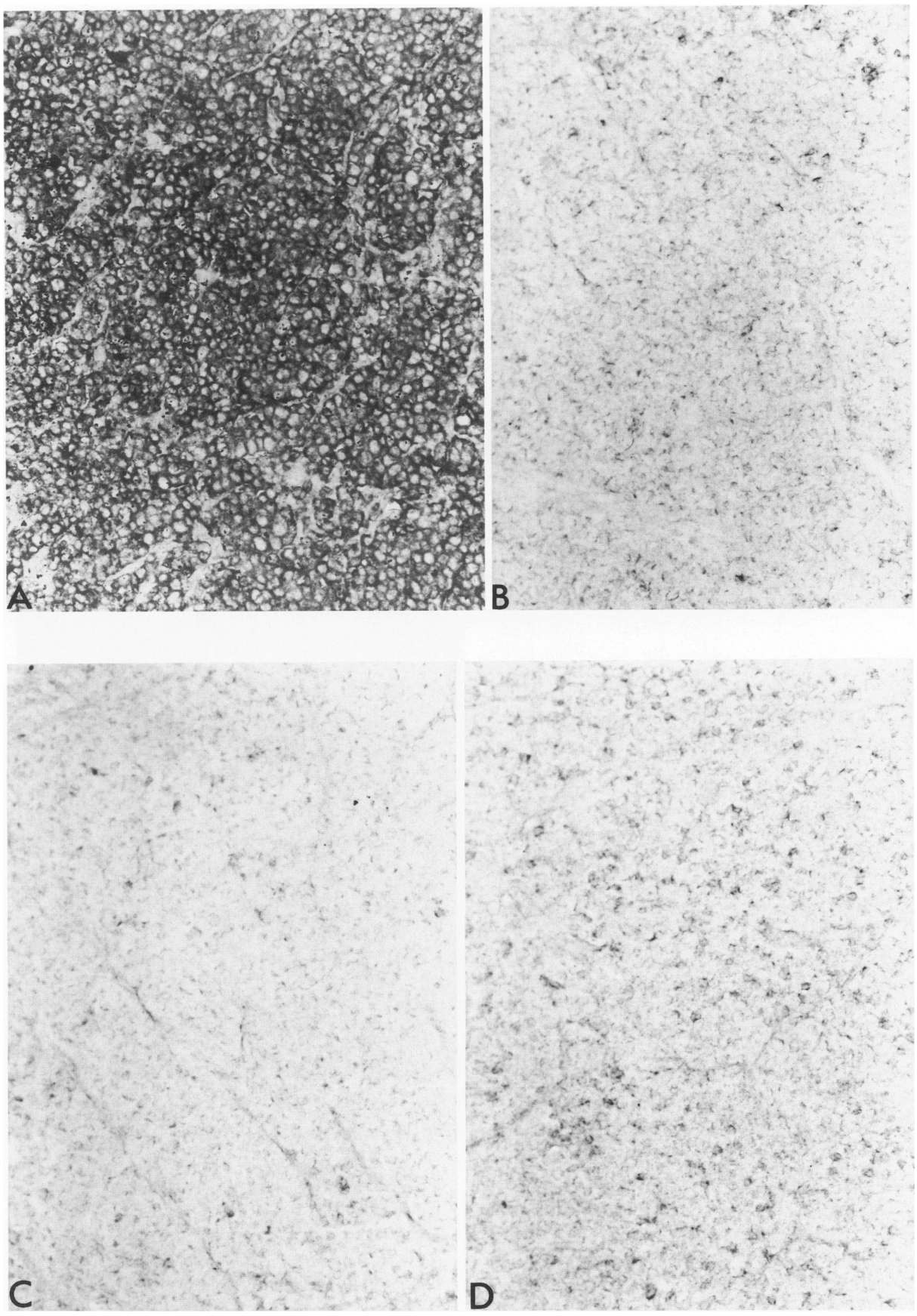

Figure 2. *Lambda protein-expressing case of multiple myeloma, paraffin sections.* **A** *shows the results of* in situ *hybridization with a cocktail of biotinylated oligonucleotide probes for lambda mRNA.* **C** *shows a similar study with the preaddition of ribonuclease.* **B** *shows results using a single biotinylated probe for lambda mRNA along with an excess of unlabeled probe.* **D** *shows results using a cocktail of five biotinylated oligonucleotide probes for kappa mRNA (200×).*

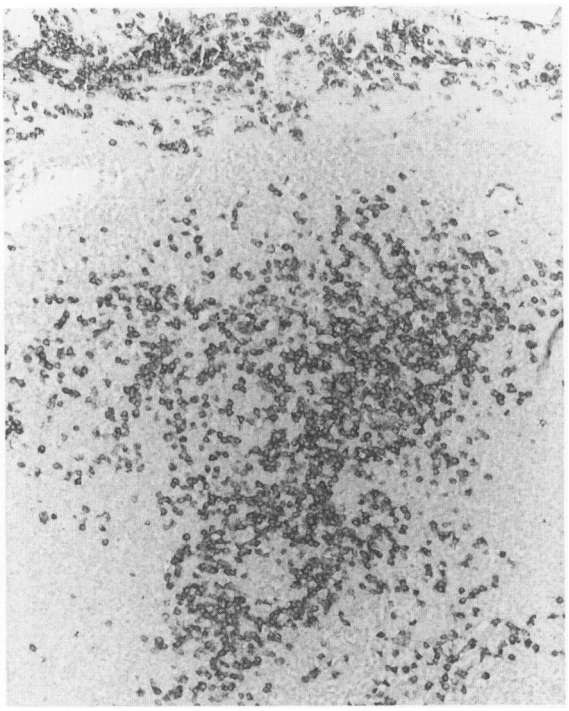

Figure 3. *Reactive lymph node, paraffin section. Many plasma cells are identified in the capsule (at top) and in the interfollicular areas.* In situ *hybridization study using a cocktail of biotinylated probes for both kappa and lambda mRNA (200×).*

ization mixture. Frozen-section slides were dehydrated in alcohols and hybridized directly without digestion or prehybridization steps. The sections were washed twice in 2× SSC for 15 minutes each at room temperature, and twice in 1× SSC for 15 minutes each at room temperature.

Detection Procedures

When hybridization was performed using biotin-labeled probes, the sections were incubated in a solution of 1% (w/v) bovine serum albumin in TRIS-saline-Brij (0.1 mol/l TRIS-Cl pH 7.5, 0.1 mol/l NaCl, 5 mmol/l $MgCl_2$, 0.25% [v/v] Brij 35) solution with a 1:500 dilution of avidin-alkaline phosphatase conjugate (Dako, Santa Barbara, CA), for approximately 2 hours at 37°C. Then the sections were subjected to three 3-minute washes of TRIS-saline-0.01% (v/v) Triton X-100 and one 3-minute wash in TRIS-saline, pH 9.5, incubated in McGadey's substrate[18] (0.33 mg/ml nitroblue/tetrazolium chloride and 0.16 mg/ml 5-bromo-4-chloro-3-indoylphosphate in TRIS-saline, pH 9.5, all from Sigma) for 30 minutes to 2 hours, briefly washed in distilled water, air dried, and covered with cover slips. The slides were examined with and without a light hematoxylin counterstain. A brown or blue-brown color over background levels was considered a positive reaction.

When hybridization was performed with 35S-labeled probes, autoradiography was performed.[8] Briefly, the sections were dehydrated in graded ethanol solutions containing 0.3 mol/l ammonium acetate and air dried. The slides were coated with an overlay of Kodak NTB2 emulsion (Rochester, NY) diluted 1:1 with 0.6 mol/l ammonium acetate at 45°C in a dark room. The slides then were dried and placed in a lightproof box containing dessicant for 3 days to 3 weeks. The emulsion was developed using Kodak D-19 developer for 4 minutes, briefly washing in water, fixing with Kodak Rapidfix for 5 minutes, and rewashing in water for 15 minutes (all at 15° to 19°C), and the slides were counterstained with hematoxylin and eosin. The slides were assessed qualitatively; cases with clear deposition of grains in excess of background levels and control slides were considered positive.

Results

The probes were initially tested on formalin-fixed paraffin sections from one case each of kappa- and lambda-positive multiple myeloma. With both biotinylated and 35S-labeled probes, cytoplasmic reactivity was obtained when a mixture of kappa gene sequence probes were applied to the case expressing kappa light chain, while no reactivity was found when applied to the case expressing lambda light chain (Figures 1 and 2). Similarly a positive reaction was obtained when a mixture of lambda gene sequence probes was applied to the case expressing lambda light chain, while a negative result was obtained on the case expressing kappa light chain (Figures 1 and 2). When single probes were used, the signal was slightly weaker, but similar results were still obtained. When a ribonuclease step was added, the labeled probe was omitted, or an excess of unlabeled probe was added to the labeled probe in the hybridization mixture, the staining pattern was abrogated (Figure 2). In addition, no staining of either case was seen using oligonucleotide probes that were the same sense as the light-chain mRNA using either 35S-labeled or biotinylated probes.

The probe cocktails then were tested on formalin-fixed paraffin sections from a variety of normal and reactive tonsils and lymph nodes. Strong reactivity of plasma cells was observed in each case, both in the paracortical areas and in the capsule (Figure 3). In addition, approximately, 10% to 40% of germinal center cells were labeled, although usually somewhat less intensely than were plasma cells (Figure 4). In cases in which immunoblasts were evident histologically, there was labeling of a proportion of these cells as well (Figure 5). There was no demonstrable light-chain gene expression within mantle zone lymphocytes, small lymphocytes in the paracortical areas, or cells within the sinuses, including cells with a 'monocytoid' ap-

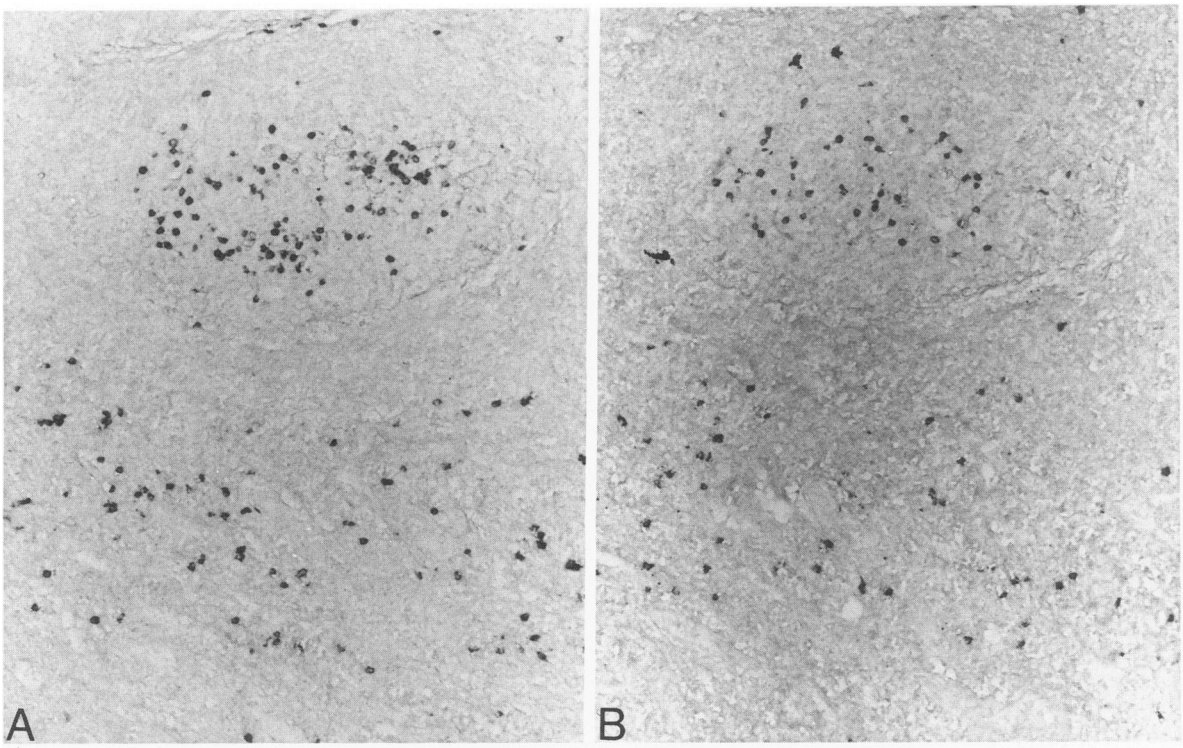

Figure 4. *Reactive lymph node, paraffin sections.* **A** *shows the results of an* in situ *hybridization study using biotinylated probes for kappa mRNA, while* **B** *shows the results of a similar study using biotinylated probes for lambda mRNA. The germinal center in the top half of the photomicrograph shows scattered positive cells in both studies. Occasional interfollicular cells (probably plasma cells) are also positive (200×).*

pearance. Cells with the morphologic appearance of histiocytes, whether within germinal centers, the paracortical regions, or within sinuses, were not labeled.

An identical pattern of staining was observed in hybridization studies performed on frozen sections. However nonspecific staining of blood vessels and lymphoid cells, particularly in the mantle zone and interfollicular regions, was observed when prehybridization was performed, which was thought to be due to endogenous alkaline phosphatase reactivity. Because addition of levamisole did not completely inhibit the staining, the prehybridization step was omitted in further frozen section studies, with elimination of the problem and little change in the sensitivity of the procedure. Nonspecific staining of lymphoid cells due to endogenous biotin or alkaline phosphatase was not a problem in paraffin section studies. In both frozen and paraffin section studies using 35S-labeled probes, nonspecific staining of eosinophils, due to binding to proteins in eosinophilic granules, was observed. This staining was readily recognized as artifactual and easily distinguished from true labeling of lymphoid cells.

In situ hybridization studies were performed next on paraffin-embedded tissue sections from a series of 103 other plasma cell and lymphoid neoplasms. Most cases had been fixed in neutral buffered formalin, with a few cases fixed in B5. Better results were obtained in the formalin-fixed cases because there was a background of nonspecific staining in several of the B5-fixed cases when biotinylated probes were used. However, positive results were obtained with both fixatives. In addition to the two initial cases of multiple myeloma described above, five additional cases of plasma cell neoplasms were studied (Table 1). All five cases showed the appropriate expression of one, but not the other, light-chain mRNA, including one case in which the demonstration of immunoglobulin protein was equivocal and one case that showed absent immunoglobulin protein as determined by conventional antibody studies also performed on paraffin sections (Figure 6).

Using an 35S-labeled solitary oligonucleotide probe for each light chain, 28 of 93 cases (30%) of non-Hodgkin's lymphoma studied showed light-chain mRNA restriction by *in situ* hybridization studies (Table 2). Fewer cases, 19%, showed light-chain mRNA restriction when a hybridization mixture of five biotinylated probes for each light chain was used. Ninety-one of the ninety-three cases (98%) showed readily detectable mRNA by studies with the poly T probe, with a consistent, strong staining in most cases; in a minority of cases, strong staining was only present in regions of the tissue. With both 35S-labeled and biotinylated probes, most cases also showed a scattered polyclonal population of plasma cells. When light-

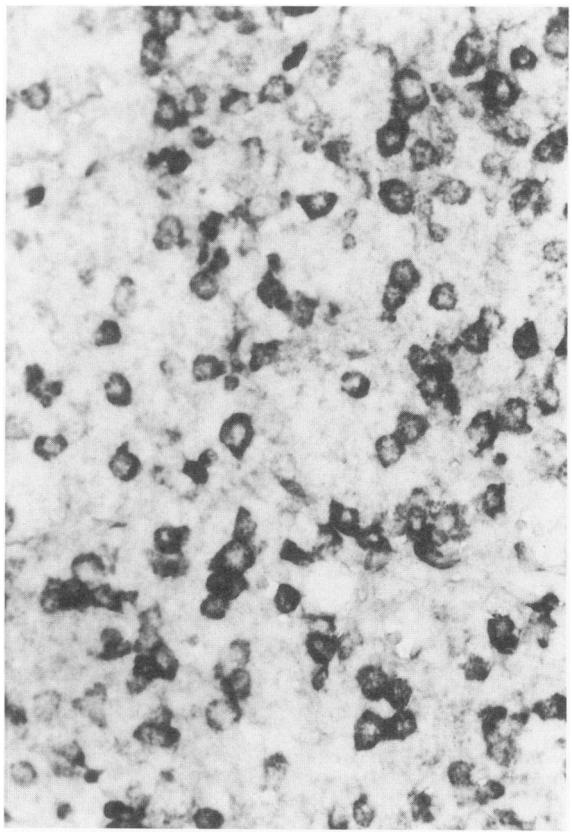

Figure 5. *Case of acute infectious mononucleosis, which histologically showed sheets of immunoblasts, paraffin section. This* in situ *hybridization study using biotinylated probes for kappa mRNA shows many positive cells. The corresponding lambda mRNA study showed a similar pattern (400×).*

chain restriction had been demonstrated by conventional antibody studies and *in situ* hybridization studies, the same light chain was present in each case. Twenty-nine percent of cases of small lymphocytic lymphoma exhibited light-chain mRNA restriction, including three of four cases exhibiting lymphoplasmacytic morphologic features. Thirty-seven percent of cases of follicular lymphoma showed light-chain mRNA restriction by *in situ* hybridization studies. Interestingly, in two cases, only the interfollicular component of the lymphoma was labeled, while the neoplastic follicles were unstained (Figure 7). Twenty-seven percent of cases of B-lineage diffuse large cell lymphoma showed light-chain mRNA restriction; neither of two cases showing a plasmacytoid morphology were positive. Overall three cases of immunoglobulin protein-negative malignant lymphomas, including one follicular lymphoma and two diffuse large cell lymphomas, showed light-chain mRNA, of the nine cases studied. Finally five cases of typical Hodgkin's disease were studied and none showed labeling of the Reed–Sternberg cell and variants. Similar to the cases of non-Hodgkin's lymphoma, there was polyclonal labeling of plasma cells in each case.

Discussion

This report describes a methodology for the *in situ* detection of kappa and lambda light-chain mRNA in paraffin-embedded tissue sections using radiolabeled and biotinylated oligonucleotide probes. The specificity of the analysis was validated in several ways. First mock hybridization studies omitting probe failed to demonstrate staining of cells. Second addition of ribonuclease abrogated the signal. Third oligonucleotide probes constructed to be the same sense as the nucleic acid sequences for the light-chain mRNA did not demonstrate detectable hybridization. Fourth competition experiments using an excess of unlabeled probe abolished the staining reaction of the labeled probe. Fifth identical results were obtained with 35S-labeled probes and biotinylated probes, making unlikely the possibility that the results were an artifact of the detection process. Sixth the results obtained by *in situ* hybridization in plasma cell neoplasms matched those of conventional immunohistochemical studies using antibody detection of light-chain protein. Finally the pattern of staining in benign lymph nodes and tonsils showed the expected pattern of reactivity, with strongest expression in plasma cells, and somewhat lesser staining of germinal center cells and immunoblasts. One discrepancy noted was the lack of staining of mantle zone cells in normal germinal centers by *in situ* hybridization. This was probably a result of the low number of copies of light-chain mRNA in this population of cells that was below the threshold of detection by this methodology. This probably represents an intrinsic property of these cells and not an artifact of tissue processing because mantle cells also were unstained in parallel studies performed on paraformaldehyde-fixed frozen sections.

We chose to use oligonucleotide probes rather than longer DNA probes or RNA probes. DNA probes of longer length, although widely available, are not optimal for *in situ* hybridization studies, due mostly to poor tissue penetration, even after prolonged protease pretreatment of the tissue sections.[8] In addition, longer DNA probes are also less easily removed, thus requiring more rigorous washing steps, and result in higher backgrounds as compared to oligonucleotide probes. Finally control sequences, such as a complementary sequence probe, usually are not available and cannot be prepared easily. RNA probes may have a higher sensitivity than oligonucleotide probes,[9] but may not be useful in a practical setting given the need for great care in handling the probes and the technical difficulty of the hybridization procedure.

This methodology should prove to be a practical method for the detection of plasma cells and their neoplasms in paraffin-embedded tissue sections, either on its own or as a complement to more conventional antibody-based immunohistochemical studies. The current study,

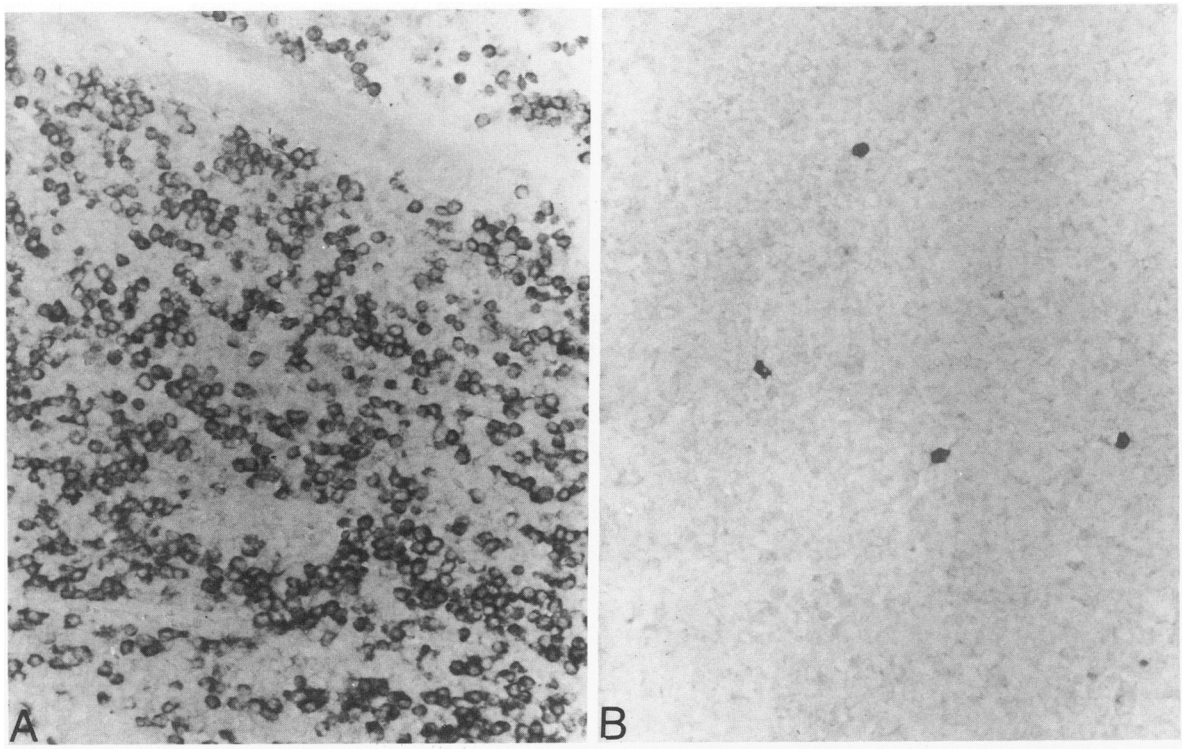

Figure 6. *Case of multiple myeloma negative for immunoglobulin protein by paraffin-section immunoperoxidase studies, paraffin sections.* **A** *shows the results of an* in situ *hybridization study using biotinylated probes for kappa mRNA, while* **B** *shows the results of a similar study using biotinylated probes or lambda mRNA. Most, but not all, tumor cells show an intense positive reaction for kappa mRNA (200×).*

as well as others, has demonstrated that *in situ* hybridization studies are useful in the detection of mRNA in paraffin sections.[10–13] Similar to the findings in our study, Akhtar et al[13] also consistently demonstrated light-chain mRNA in cases of suspected multiple myeloma. The fact that biotinylated probes can provide sufficient sensitivity for these analyses eliminates the need for radioisotopes and their dangers and expense.[14] In addition, biotinylated probes have a long shelf-life and results can be obtained within 1 day. The procedure is relatively inexpensive;

Table 2. *Results of* In Situ *Hybridization Studies for Light-chain mRNA in Paraffin Sections of Cases of Plasma Cell Neoplasms and Malignant Lymphomas*

	Total number	Number positive with 35S-labeled probe (One oligonucleotide)	Number positive with biotinylated probe (Five oligonucleotides)	Number positive with biotinylated positive control poly T probe
Plasma cell neoplasms	7	7	7	ND†
Ig^{+}*	5	5	5	ND
Ig^{-}	2	2	2	ND
Non-Hodgkin's lymphomas	93	28	18	91
Small lymphocytic	37	10	5	36
Ig^{+}	35	10	5	34
Ig^{-}	2	0	0	2
Follicular	30	11	5	29
Ig^{+}	28	10	5	27
Ig^{-}	2	1	0	2
B-lineage diffuse large cell	26	7	8	26
Ig^{+}	21	6	6	21
Ig^{-}	5	1	2	5
Hodgkin's disease	5	0	0	ND

* Immunoglobulin studies using antibodies performed on paraffin sections for plasma cell neoplasms and performed on frozen sections or on cell suspensions for non-Hodgkin's lymphomas and Hodgkin's disease.

† Not done.

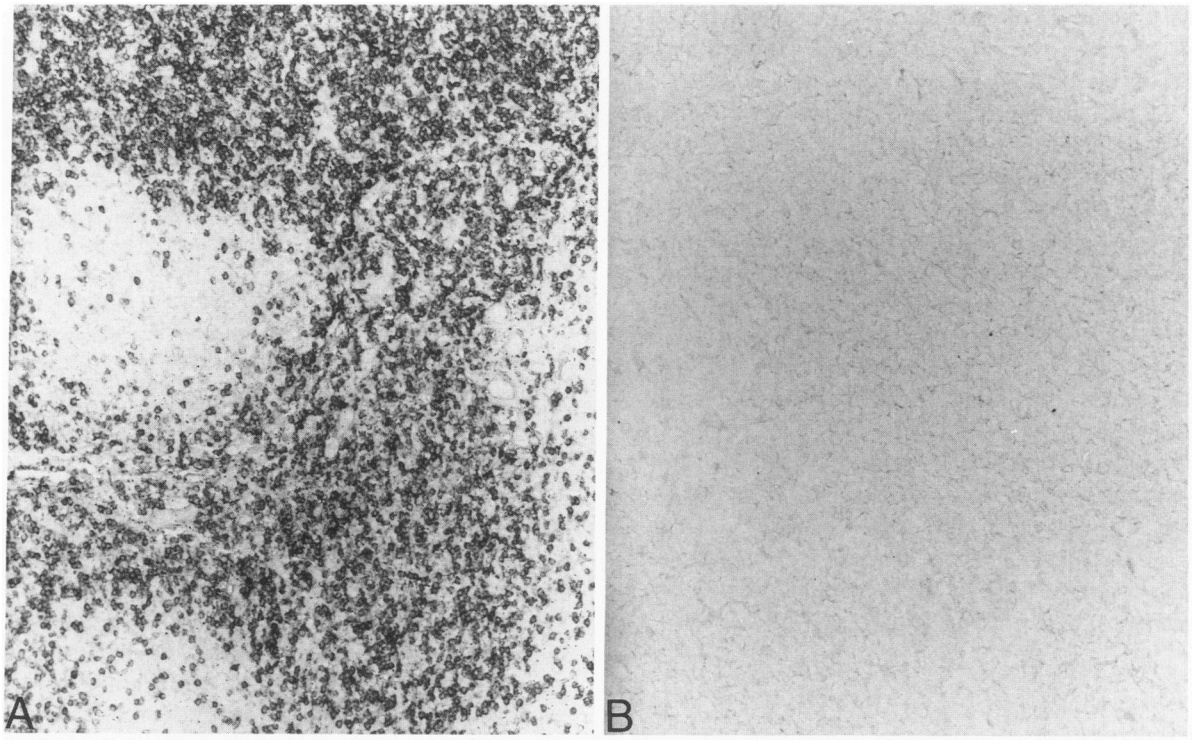

Figure 7. *Follicular lymphoma, paraffin sections.* **A** *shows the results of an* in situ *hybridization study for kappa mRNA, while* **B** *shows the results of a similar study for lambda mRNA. Lymphoma cells in the interfollicular areas are positive, while the neoplastic germinal centers are negative (200×).*

aside from the initial costs of obtaining the appropriate probes, the methodology is comparable to conventional immunohistochemical procedures. There is one potential pitfall specifically associated with the alkaline-phosphatase detection system that we have used: endogenous alkaline phosphatase within tissues may conceivably give rise to false-positive results. Occasionally this was observed in frozen section studies in which the prehybridization step was included; these artifacts appeared in the form of blood vessel staining and light staining of mantle zone and interfollicular lymphocytes. For this reason, we generally omitted the prehybridization step when performing analyses on frozen sections. The nonspecific staining can be readily recognized by examination of blood vessels for reactivity, and may potentially be avoided by the addition of levamisole to the wash solutions to inhibit the endogenous alkaline phosphatase.

Unfortunately the current protocols are not sufficiently sensitive to consistently identify light-chain mRNA in formalin-fixed paraffin-embedded sections of most cases of B-lineage non-Hodgkin's lymphomas. In the current study, we detected light-chain mRNA restriction in approximately 30% of cases of non-Hodgkin's lymphomas using a single 35S-labeled probe for each light chain and about 20% of cases of non-Hodgkin's lymphoma using a mixture of five biotinylated probes for each analysis. Although the studies with the poly T probe showed the presence of large quantities of detectable mRNA in most cases, we cannot preclude the possibility of selective or partial degradation of mRNA. It is also possible that a greater degree of sensitivity may be obtained using a greater number of probes in the hybridization mixture. For example, Akhtar and colleagues[13] used cocktails of up to 15 oligonucleotide probes compared to the five used in the current study. Nonetheless the results we obtained in paraffin sections are still better than most,[6,7] but not all,[5] comparable studies using antibodies to detect immunoglobulin protein.

It is also unclear why we could consistently detect light-chain mRNA in reactive germinal centers yet only achieve positive results in less than 40% of cases of follicular lymphoma. Again selective or partial degradation of mRNA is a possibility. Alternatively perhaps most follicular lymphomas are frozen in a maturation stage before the bulk of immunoglobulin mRNA synthesis occurs. This was probably the case in the two follicular lymphomas in which the neoplastic follicles lacked light-chain mRNA expression, while the neoplastic cells in the interfollicular areas strongly expressed light-chain mRNA. Interestingly we encountered some cases of apparently immunoglobulin protein-negative B-lineage diffuse large cell lymphoma in which we detected light-chain mRNA. This suggests that, at least in these few cases, either a relative or absolute defect in immunoglobulin synthesis in these

lymphomas is located at the translational, and not transcriptional, level. Our technique provides a way to easily assess the clonality of such tumors.

The greatest potential for this methodology may be as a model for future *in situ* hybridization studies for mRNA detection in formalin-fixed paraffin sections using oligonucleotide probes. The procedures used may be applicable to the detection of other mRNA species of interest and should be easily transferred if the nucleotide sequence for the gene of interest is known and sufficient specific mRNA is present within the tissues to be studied. Because oligonucleotide probes are used, they can be custom synthesized for each gene of interest and, once prepared, should have properties similar to the oligonucleotides used in the current study, with only minor modifications to the actual *in situ* hybridization procedure required. Thus the expression of a wide range of genes may be studied in frozen and paraffin sections either as a complement to antibody studies of protein expression or alone, if the relevant antibodies are not yet available.

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