VIII. APPENDIX

A. Cell lines

1. U-937

The U-937 was established by Sundstrom and Nilsson (1976), from malignant cells obtained from the pleural effusion of a 37-year-old Caucasian male with diffuse histiocytic lymphoma. This is a near triploid human cell line. The modal chromosome number was 58, occurring in 70% of cells. They included: der(3)t(1;3)(q21; q27), st(1q14q), t(1p13q), der(5) t(1;5)(p13;q31), $2q^{+}$,t(6p12q), and 12-14 others. All markers had a single copy per cell, which generally applied to N1-N3, N5-N6, N10-N13, and N16. The X chromosome had two copies per cell and the DM's was not detected. The U-937 is one of only a few human cell lines that still expresses many of the monocyte-like characteristics exhibited by cells of histiocytic origin. The cells bear receptors for Fc and C3, phagocytose antibody-coated erythrocytes and latex beads, and stain strongly with non-specific lysozyme. In addition, the cells lack EB virus-related antigens and both surface and intracellular immunoglobulins. Studies since 1979 have shown that U-937 cells can be induced to terminal monocytic differentiation by a supernatant from human/mixed lymphocyte cultures, phorbal ester, vitamin D3 (Olsson et al., 1983), and retinoic acid, γ interferon and TNF (Andersson et al., 1985).

2. K-562

The continuous cell line K-562: the human chronic myelogenous leukemia, was established by Lozzio and Lozzio from the pleural effusion of a 53-year-old female with chronic myelogenous leukemia in terminal blast crises. The cell population has been characterized as highly differentiated and of the granulocytic series. Studies conducted by Andersson et al. (1979) on surface membrane properties led to the conclusion that the K-562 was a human erythroleukemia line. The effect of inducers on sublines derived from the original K-562 line has been reviewed by Koeffler and Golde (1980). More recent studies indicate that K-562 blasts are multipotential, haematopoietic malignant cells that spontaneously differentiate into the recognizable progenitors of the

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erythrocytic, granulocytic and monocytic series (Lozzio and Lozzio, 1981).

The K-562 cell line has attained widespread use as a highly sensitive *in vitro* target for the natural killer assay. The karyological studies on various K-562 sublines have been classified into three groups (A, B, C) by Dimery et al. (1983), with the Chromosome Frequency Distribution of 50 cells:2n=46. The stemline chromosome number is triploid with the 2S component occurring at 4.2%. Fifteen markers (M1 to M15) occurred in nearly all S metaphases. Spontaneous non-specific discentrics occurred, but rarely. Unstable markers were also rarely seen. The X was disomic, N9 was nullisomic and the morphology was lymphoblast-like.

3. KG-1

The KG-1 cell line was established by Koeffler and Golde (1978). A bone marrow aspirate was obtained from a 59-year-old Caucasian male with erythroleukemia that evolved into acute myelogenous leukemia. The cells were cultured in suspension, using alpha minimum essential medium containing 20% fetal bovine serum and 10^{-4}M α-thioglycerol. After 24 days in culture, the cells were actively proliferating. KG-1 grows predominantly as single cells with numerous small, irregular aggregates of 8 to 14 cells interspersed throughout the static suspension. Morphologically, KG-1 cells resemble acute myelogenous leukemia, showing considerable pleomorphism with a predominance of myeloblasts and promyelocytes. A small percentage of the cells are mature granulocytes, and occasionally macrophages and eosinophils are also present. The cells stained heavily with ASD chloroacetate esterase and 1-2 percent of them stained with peroxidase and Sudan black B.

A unique characteristic of KG-1 cells is their responsiveness to the colony-stimulating factor measured by the formation of colonies in a soft-agar culture. The KG-1 cells lack specific markers for lymphocytic cells. They have no surface immunoglobulins or Epstein-barr virus-associated antigens, but they do express the human Ia-like or DR antigen (Koeffler and Golde, 1980). KG-1 differentiates, without DNA replication, into non-dividing macrophages when exposed to phorbal esters. The karyological studies indicate the Chromosome Frequency Distribution 50 cells:2n=46. The stemline chromosome number is near-diploid, with the 2S component occurring at 1.8%. Five markers (constutive markers) were common to most, if not all, metaphases analyzed. The modal chromosome number is

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47 (all 46 plus a small metacentric chromosome which is smaller than the G1 group chromosome). Normal chromosome 5, 7, 8, 12, and 17 were monosomic, and others were disomic. The Y chromosome is detected in the Q-banded preparations. KG-1 has the myeloblast-like morphology.

4. HL-60

HL-60 is a promyelocytic cell line established by Collins et al. (1977). Peripheral blood leukocytes were obtained by leukopheresis from a 36-year-old Caucasian female with acute promyelocytic leukemia. The cells were cultured in suspensions in the presence of a conditioned medium from human embryonic lung cultures. Subsequently, it was found that continued growth of the cells did not required the conditioned medium. The predominant cell population that has been maintained consists of neutrophilic promyelocytes with prominent nuclear/cytoplasmic asynchrony. Up to 10% of the cultured cells differentiate spontaneously beyond the promyelocytic stages and the proportion is markedly enhanced by polar-planar compounds such as DMSO. A wide variety of other compounds, including butyrate, hypoxanthine, TPA, actinomycin D and retinoic acid also induce differentiation.

The HL-60 cells lack specific markers for lymphoid cells, but they express surface receptors for Fc fragments and complements (Gallagher et al., 1979). They exhibit phagocytic activity and responsiveness to chemotactic stimuli. HL-60 cells form colonies in semi-solid media and produce subcutaneous myeloid tumors in nude mice. The stemline chromosome number is pseudodiploid with the 2S component occurring at 6.2%. Five markers (M2 to M6) were common to the most S metaphases. DM's, which varied in number per cell, occurred in all metaphases karyotyped. HSR chromosomes were not detected. The Chromosome Frequency Distribution 50 cells:2n=46 and themorphology is lymphoblast-like.

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5. HEp-2

The HEp-2 cell line was established by Moore et al. (1955) from tumors that had been produced in irradiated-cortisonized weanling rats after being injected with epidermoid carcinoma tissue from the larynx of a 56-year-old Caucasian male (Toolan, 1954). The *in vitro* isolation was accomplished in each of several mixtures of bovine amniotic fluid, embryo

extracts, human and horse sera, and balanced salt solution. The epithelial-like cells subsequently grew well in several types of culture media. It is a hardy cell line, which resists temperature, nutritional and environmental changes without lost of viability.

The HEp-2 line supported the growth of 10 of 14 arboviruses and a measles virus. It has been used for experimental studies of tumor production in rats, mice, embryonated eggs and volunteer terminal cancer patients.

The karyological studies indicate the Chromosome Frequency Distribution 47 cells:2n=46, which occasionally polyploids. Several marker chromosomes were observed along with frequent minutes, and often, 2 large chromosomes with subterminal centromeres. The morphology is epithelial-like.

B. Reagents for indirect immunofluorescent technique

1. 1% bovine serum albumin in phosphate buffered saline pH 7.2 with 0.02% azide (1% BSA-PBS azide)

Bovine serum albumin fraction IV	1.0	gm
Phosphate buffered saline (PBS) pH 7.2	80	ml

   - Mixed until well dissolved and added 10% sodium azide to make 0.02% concentration, finally top up to 100 ml with PBS.
   - Stored at 4°C.
2. Phosphate buffered saline (PBS) pH 7.2

NaCl	8.0	gm
KCl	0.2	gm
Na_2HPO_4	1.15	gm
KH_2PO_4	0.2	gm

   - Added distilled water 800 ml and stirred on magnetic stirrer until well dissolved.
   - Adjusted the pH to 7.2 with 1N HCl.
   - Filled up to 1000 ml with distilled water and stored at room temperature.

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C. Reagent for Flow cytometrical analysis

1. Fixative in PBS

37% formaldehyde	0.675	ml
PBS pH 7.2	49.325	ml

- Mixed well and stored at room temperature.

D. Reagents for isolation of human blood cells and cell culture

1. Phosphate buffered saline (PBS) pH 7.2

NaCl	8.00	gm
KCl	0.20	gm
Na_2HPO_4	1.15	gm
KH_2PO_4	0.20	gm

- Dissolved all ingradients in about 800 ml distilled water.
- Adjusted pH to 7.2 with 1N HCl or 1N NaOH.
- Filled up to 1000 ml with distilled water and stored at room temperature.

2. Ficoll-Hypaque solution

2.1 9% Ficoll

Ficoll	22.5	gm
Distilled water	200	ml

- Dissolved Ficoll in warm distilled water by stirring on magnetic plate.
- Top up with distilled water to 250 ml.

2.2 34% Hypaque

50% Hypaque	68.0	ml
Distilled water	32.0	ml

- Added Hypaque into distilled water and mixed well.

2.3 Ficoll-Hypaque solution

9% Ficoll	240	ml
34% Hypaque	100	ml

- Sterilized by filtration and stored at 4 °C.

3. RPMI medium
   - Added RPMI 1640 medium powder to distilled water with gentle stirring.
   - Rinsed out inside of package to remove all traces of powder.
   - Added 2.0 gm of $NaHCO_3$.

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- Added gentamycin and amphotericin B to final concentration of 40 μg/ml and 2.5 μg/ml, respectively.
- Added distilled water to about 900 ml, mixed well and adjusted pH to 7.2 with 10% acetic acid.
- Filled up with distilled water to 1000 ml.
- Sterilized by filtration and stored at 4 °C.

4. 10% FCS-RPMI 1640
   - Mixed 10.0 ml of heat inactivated fetal calf serum and 90.0 ml RPMI-1640 together by sterlile technique in the laminar air flow and kept at 4 °C.
5. Ammonium chloride Tris (NH_4Cl- Tris pH 7.2)
   5.1 NH_4Cl (0.83% w/v in H_2O)

NH_4Cl	0.83	gm
H_2O	100	ml

   5.2 Tris (2.06% w/v in H_2O, pH 7.65)

Tris-base	0.206	gm
H_2O	10	ml

   - Mixed 9 parts of 5.1 together with 1 part of 5.2
   - Adjusted pH to 7.2 and stored at room temperature.
6. Lysing solution (Hypotonic NH_4Cl)

NH_4Cl	8.29	gm
$KHCO_3$	1.00gm	
disodium EDTA	0.04	gm

   - Added 800 ml of distilled water and mixed until well dissolved.
   - Adjusted pH to 7.4 with 1N HCl, filled up to 1000 ml with distilled water and stored at 4 °C.
7. 0.5% trypan blue in normal saline

Trypan blue powder	0.5	gm
Normal saline	100	ml

   - Dissolved trypan blue in normal saline and stored at room temperature.

E. Reagents for SDS-PAGE

1. Acrylamide/Bis acrylamide 30%

Acrylamide	29.2	gm
N,N,-Bis methylene acrylamide	0.8	gm

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- Added 80.0 ml of distilled water and stirred on magnetic plate until well dissolved, then filled up to 100 ml.
- Filtered through 0.45 micron milipore to eliminate excess acrylamide.
- Kept away from direct light and stored at 4 °C.

2. 1.5M Tris-HCl pH 8.8

Tris-base	18.17	gm
Distilled water	100	ml

- Dissolved Tris-base in about 50.0 ml of distilled water.
- Adjusted the pH to 8.8 with 1N HCl.
- Filled up with distilled water to 100 ml and stored at room temperature.

3. 0.5M Tris-HCl pH 6.8

Tris-base	6.06	gm
Distilled water	100	ml

- Dissolved Tris-base in about 50.0 ml of distilled water.
- Adjusted the pH to 6.8 with concentrate HCl.
- Filled up with distilled water to 100 ml and stored at room temperature.

4. 10% w/v Sodium dodecyl sulphate (SDS)

Sodium dodecyl sulphate	1.0	gm
Distilled water	10	ml

- Dissolved SDS in distilled water and mixed well.
- Divided the solution into aliquots and stored at -20 °C.

5. 10% w/v Ammonium persulphate (APS)

Ammonium persulphate	1.0	gm
Distilled water	10	ml

- Dissolved APS in distilled water and mixed well.
- Divided the solution into aliquots and stored at -20 °C.

6. 5x Non-reducing buffer (sample buffer)

0.5M tris-HCl pH 6.8	3.0	ml
Glycerol	2.4	ml
10% w/v SDS	4.8	ml
0.5% w/v Bromphenol blue	0.6	ml
Distilled water	15.2	ml

- Mixed all ingradients together until well dissolved.
- Divided the solution into aliquots and stored at -20 °C.

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7. 5x Reducing buffer

5x Non reducing buffer	9.5	ml
2-Mercaptoethanol (2-ME)	0.5	ml

- Added 2-ME into 5x Non reducing buffer and mixed well .
- Divided the solution into aliquots and stored at -20 °C.

8. 5x Running buffer pH 8.3

Tris-base	7.8	gm
Glycine	35.0	gm
SDS	2.5	gm

- Added about 300 ml of distilled water and mixed well.
- Adjusted the pH to 8.3 with 1N HCl or 1N NaOH.
- Filled up to 500 ml with distilled water and stored at room temperature.

9. Gel staining solution

Coomasie Brillient blue 250R	0.25	gm
Absolute methanol	40	ml
Glacial acetic acid	10	ml

- Mixed all reagents together and filled up to 100 ml with distilled water.
- Stored at room temperature.

10. Gel destaining solution

Absolute methanol	40.0	ml
Glacial acetic acid	10.0	ml

- Added 50.0 ml of distilled water into the mixture of above reagents.
- Mixed well and stored at room temperature.

11. 10% Separating or resolving gel

Acrylamide/Bis acrylamide 30%	3.3	ml
1.5M tris-HCl pH 8.8	2.5	ml
Distilled water	4.0	ml
10% w/v SDS	0.1	ml
10% w/v APS	0.1	ml
TEMED	0.01	ml

- Swirled the solution gentry but thoroughly. The APS and TEMED are added to the gel solution immediately prior to pouring the gel.
- The polymerization are allowed to occur at room temperature prior to run electrophoresis.

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12. 4% Stacking gel

Acrylamide/Bis acrylamide 30%	1.3	ml
0.5M Tris-HCl pH 6.8	2.5	ml
Distilled water	6.0	ml
10% w/v SDS	0.1	ml
10% w/v APS	0.1	ml
TEMED	0.01	ml

- Freshly prepared prior to use as described in 11.

F. Reagents for Western blotting

1. Blotting buffer

Tris-base	1.5	gm
Glycine	7.21	gm
SDS	0.047	gm
Absolute methanol	200	ml

- Dissolved all ingradients in about 300 ml of distilled water and filled up to 500 ml.
- Stored at room temperature.

2. Nitrocellulose staining solution

Isopropanol	25.0	ml
Glacial acetic acid	10.0	ml
Amido Black 10B	0.1	gm

- Dissolved the ingradients all together and filled up to 100 ml with distilled water.
- Stored at room temperature.

3. Nitrocellulose destaining solution

Isopropanol	125	ml
Glacial acetic acid	50	ml

- Added distilled water to the mixture of the above ingradients to make 500 ml and stored at room temperature.

G. Reagents for immunoblotting

1. Phosphate buffered saline with 0.05% Tween (PBSTween)

PBS	1000	ml
Tween-20	0.5	ml

- Freshly prepared on the day of experimemt.

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2. 5% Skimmed milk in PBS pH 7.2

Non-fat dried milk	2.5	gm
PBS pH 7.2	50.0	ml

- Dissolved non-fat dried milk in about 30.0 ml PBS and mixed well by magnetic stirrer.
- Filled up with PBS to 50.0 ml.

3. Chemiluminescence reagents (working solution)

Reagent A	1	part
Reagent B	1	part

- Mixed together immediately prior to use.

4. Developer

Developer	75.0	ml
Distilled water	225	ml

- Mixed throughly and kept at 4 °C .

5. Fixer

Rapid fixer solution A	67.0	ml
Rapid fixer solution B	8.0	ml
Distilled water	225	ml

- Mixed throughly and kept at 4 °C .

H. Reagents for preparation of cell lysate

1. Tris lysis buffer pH 8.2

Tris-base	0.60	gm
NaCl	4.10	gm
EDTA	0.37	gm

- Dissolved Tris-base in 300 ml distilled water and adjusted the pH to 8.2.
- Added NaCl and EDTA, mixed well and filled up with distilled water to 500 ml.
- Stored at room temperature.

2. Lysis solution

Phenylmethylsulphonyl fluoride (PMSF stock 100 mM in acetone)	0.1	ml
Iodoacetamide (stock 0.5 M)	0.1	ml
Nonidet p-40	0.1	ml
Tris lysis buffer pH 8.2	9.6	ml

- Mixed well and divided the solution into aliquots.
- Stored at -20 °C.

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I. Reagents for cell proliferation assay

1. [^{3}H] thymidine 0.2 μCi per 50 μl

[^{3}H] thymidine (Amersham)	0.120	ml
10% FCS-RPMI	30.0	ml

- Mixed well and stored at 4 °C.

2. Liquid scintillation fluid

2,5-diphenyloxazole (PPO)	10.0	gm
Dimethyl POPOP	0.25	gm
Toluene	2.5	L

- Dissolved the two reagents in toluene and stored at room temperature.

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IX. CURRICULUM VITAE

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