PERCENTAGE OF POSITIVELY-LABELLED CELLS

Specimen	CD45 pan leucocyte	CD3* pan T-cell	CD4 T-helper cells	CD8 cytotoxic T-cells	HLA-DR activated cells
Pooled control fascia	<0·5	<0·5	NT	NT	1·5
Patient					
1	30	27	NT	NT	25
2	16	15	1·9	2·7	29
3	25	23	1·3	4·1	15
4	21	26	2·8	5·0	30
5	26	26	2·2	5·0	30
6	18	26	4·3	6·0	27
7	21	28	NT	NT	26
8	37	25	NT	NT	26
9	14	30	NT	NT	NT
10	15	27	NT	NT	NT
11	22	17	NT	NT	NT
12	14	22	NT	NT	NT
13	34	23	1·8	NT	NT
14	30	29	NT	NT	26

Results >1% rounded to 2 significant figures NT = not tested.
*Several antibody combinations included CD3; results are mean values derived from all available CD3 results for that specimen.

subset of T-lymphocytes, such as the recently described population of "double-negative" T-cells found in epidermis;[8] this population needs to be defined. The increased frequency of HLA-DR-positive cells in Dupuytren's disease indicates expression of major histocompatibility complex (MHC) class II molecules, and the potential ability of these cells to present antigen to T-lymphocytes. HLA-DR is generally recognised as an indicator of cell activation, and activated T-cells also release cytokines that upregulate expression of MHC class II proteins encoded by genes of the HLA-DR locus. These findings are consistent with the inappropriate expression of fibroblast-stimulating cytokines reported in this disorder.[7]

Although T-lymphocytes probably act as mediators in the pathogenesis of Dupuytren's disease, we do not know whether they act as regulator or effector cells; nor has any specific antigen been identified. The precise role of the T-cells requires definition, and further studies are underway to characterise the other cells present in diseased issue (eg, macrophages and their interactions with lymphocytes). Dupuytren's disease might be triggered by the interaction of environmental factors with the primary genetic defect. One hypothesis consistent with our findings is that the defect might occur in genes coding either for MHC proteins or for T-cell receptor proteins. T-lymphocytes are certainly implicated in the pathogenesis of autoimmune disorders, several of which are associated with Dupuytren's disease. The HLA-antigen status of Dupuytren's patients has been recorded,[8] and at least one possible pattern of expression has emerged. Type I diabetes is associated with HLA-DR3 and DR4, and up to 30% of diabetics also have Dupuytren's disease. Conversely, there is a negative correlation between Dupuytren's disease and rheumatoid arthritis, a condition known to have strong association with HLA-DR4. Dupuytren's disease arises in 36% of patients infected with HIV,[4] and subcuticular fibrosis nodules with histological appearances similar to Dupuytren's nodules have been reported in simian acquired immune deficiency syndrome.[9] In alcoholic hepatic cirrhosis, T-cells are thought to support the production of cytokines by liver macrophages and these factors then regulate the fibrotic process.[10] Finally, the onset of Dupuytren's disease following injury in genetically-susceptible individuals[5] might be related to the large pool of activated T-cells and macrophages present in the wound.

The subcutaneous nature of the disorder allows its natural history to be followed with ease; if we can confirm that the disease is caused by cell-mediated immune mechanisms, medical therapy might be developed as an adjunct or alternative to surgery, allowing a more conservative surgical approach with the prospect of reduced postoperative morbidity and recurrence.

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Coelocentesis: a new technique for early prenatal diagnosis

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Chorionic villus sampling and amniocentesis have disadvantages. In 100 women undergoing termination of pregnancy, coelomic fluid was successfully aspirated in 96% of cases at 6–10 weeks' gestation, 42% at 11, and 10% at 12 weeks. Cytogenetic analysis always failed with coelomic fluid, but fetal sexing was always successful with fluorescence in-situ hybridisation and polymerase chain reaction, and the results agreed with those obtained from chorionic villi and amniotic fluid in all cases. Coelocentesis may be suitable for prenatal diagnosis in the first trimester.

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Prenatal diagnosis in the first trimester provides early reassurance to most mothers that their fetus is not affected by the genetic disorder under investigation. To those with an affected fetus, early diagnosis provides the option of early termination. Embryonic tissues suitable for early prenatal diagnosis are obtained by chorionic villus sampling (CVS) or early amniocentesis. However, CVS may be associated with a higher risk of pregnancy loss compared with second-trimester amniocentesis.[1] Furthermore, the possible association between CVS at less than 10 weeks and fetal limb reduction defects is likely to confine its application to pregnancies beyond 10 weeks.[2,3] Amniocentesis can be done from 10 weeks onwards, before which the number of viable cells is small and there is a high failure rate.[4] The safety and diagnostic accuracy of early amniocentesis remains to be determined.[5] We report a new procedure, coelocentesis, that is best done before 10 weeks. During the first 12 weeks of pregnancy the amniotic sac is surrounded by coelomic fluid in the extraembryonic coelomic cavity, which is a derivative of the extra-embryonic mesoderm.

In 100 women with normal singleton pregnancies at 6–12 weeks' gestation, written consent was obtained for coelocentesis and amniocentesis immediately before elective termination for psychological indications. The protocol was approved by the Research Ethics Committee of King's College Hospital. After the administration of general anaesthesia, transvaginal ultrasonography was used to confirm gestational age (crown-rump length) and identify the placenta and amniotic membrane. A 20 gauge needle was introduced transvaginally into the coelomic cavity under continuous ultrasound monitoring and the fluid was aspirated. In all cases the needle tip was located. The procedure was not associated with alterations in fetal heart rate and there was no evidence of haemorrhage into the coelomic cavity. Amniocentesis was then done with a new needle. Subsequently, suction termination was carried out and placental tissue was collected.

Coelomic fluid was successfully aspirated in nearly all cases up to 10 weeks' gestation, but the success rate fell at later weeks (table I). The mean volume of aspirated fluid doubled between 6 and 10 weeks, and then fell. For amniocentesis, the rate of successful sampling increased with advancing gestation from 31% (4/13) at 7 weeks to 77% (10/13) at 8 weeks and 100% (68/68) at 9–12 weeks. Coelomic fluid was always bright yellow, while amniotic fluid was clear. The nature of the samples was confirmed biochemically.[6]

In 30 cases, sampled at 8–10 weeks' gestation, an attempt was made to determine fetal sex by analysing amniotic fluid, coelomic fluid, and placental tissue. 10 cases had standard cytogenetic analysis. The second 10 cases had polymerase chain reaction (PCR) with Y centromeric primers.[7] The final product was run in 1% agarose gel and stained with ethidium bromide. The remaining 10 had fluorescence in-situ hybridisation (FISH) with alpha satellite repeat probes for X and Y chromosomes (Cytocell, Lewknor, Oxfordshire). The slides were examined by fluorescence microscopy without the need for signal amplification.

TABLE I—SUCCESS RATE OF COELOCENTESIS AND VOLUME OF ASPIRATED FLUID

Gestation (wk)	Cases	Success	Volume (mL) (mean, range)
6	6	6 *(100%)*	3·1 (2·3–3·5)
7	13	12 *(92%)*	4·6 (3·6–7·1)
8	13	12 *(92%)*	5·6 (3·5–7·2)
9	18	17 *(94%)*	5·5 (3·0–7·6)
10	17	17 *(100%)*	6·1 (4·1–8·3)
11	12	5 *(42%)*	3·4 (2·2–5·1)
12	21	2 *(10%)*	3·5 (2·5–4·5)
Total	100	71 *(71%)*	4·3 (2·2–8·3)

TABLE II—CELL CULTURE, FISH, AND PCR IN FETAL SEXING

Technique (10 each)	Result	Coelomic fluid	Amniotic fluid	Placental tissue
Culture	Male	0	3	6
	Female	0	2	4
	Failed	10	5	0
FISH	Male	5	5	5
	Female	5	5	5
	Failed	0	0	0
PCR	Male	3	3	3
	Female	7	7	7
	Failed	0	0	0

Cytogenetic analysis was successful in all placental samples. However, cells failed to culture in half the amniotic fluid and in all coelomic fluid samples (table II). FISH and PCR were successful in all samples from the three compartments, with concordance in fetal sex prediction.

We have demonstrated the feasibility of coelocentesis and its potential for prenatal diagnosis before 10 weeks' gestation. Although the risks of coelocentesis in continuing pregnancies remain to be assessed, the procedure may be safer than early amniocentesis, which involves the removal of a large proportion of amniotic fluid with a potential adverse effect on fetal pulmonary development.[8] In addition, coelocentesis does not involve puncture of the amniotic membrane and therefore the risk of direct trauma to the embryo or chronic amniotic fluid leakage would be expected to be lower than with amniocentesis. Coelocentesis may also be preferable to CVS for prenatal diagnosis. Coelomic cells are derived from the extra-embryonic mesoderm, and thus there may be fewer problems with pseudomosaicism than are encountered with trophoblastic cell preparations. Additionally, coelocentesis does not involve puncture of the definitive placenta, reducing the possibility of placental vascular damage and consequent fetal abnormality.

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