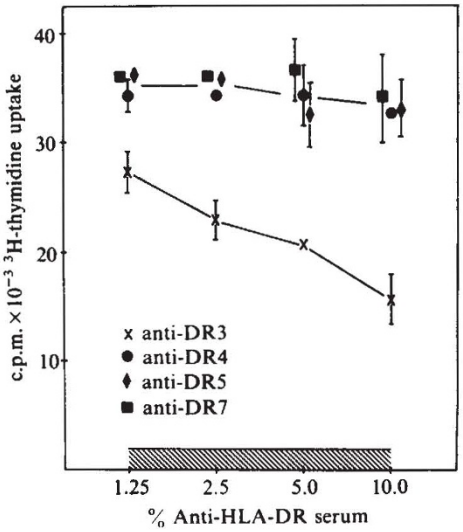

Fig. 4 Inhibition of AChR-induced proliferation (c.p.m. ± s.d.) of the autoreactive, DR3-positive long-term T-cell line (patient 1) with anti-DR3 alloantiserum. The cells were cultured as described in Fig. 1 in the presence of an optimal stimulating concentration of *Torpedo* AChR (5 μg ml^{-1}) and various concentrations of thrombocyte-absorbed DR-specific alloantisera (Biotest; anti-DR3: lot no. 111952; anti-DR4: 112042; anti-DR5: 112331; anti-DR7: 112052; all antisera were dialysed against medium before use in tissue culture). Only anti-DR3 antiserum inhibited AChR-induced proliferation. Hatched area: range of background proliferation in the absence of antigen.

Our results suggest that autoreactive inducer T cells or their precursors may initiate and/or regulate the production of myasthenogenic anti-AChR autoantibodies *in vivo*. The finding that the cells react both with *Torpedo* and human AChR suggests that antigenic determinants shared between fish and mammalian AChR at least participate in the primary autosensitization event[18,24], although our propagation protocol (using *Torpedo* AChR) was likely to select for T-cell clones cross-reactive with *Torpedo* and human AChR. The observation that the AChR-specific T cells were, in three patients, restricted either partially or exclusively to HLA-DR3 leads us to speculate that the (weak) association of certain forms of myasthenia gravis with HLA-B8/DR3 (refs 8, 9) may partially reflect the recognition by autoreactive T lymphocytes of self-AChR and, possibly, other autoantigens[25] in the context of DR3-related determinants. Purified autoimmune human T cells seem to represent an ideal tool for further studies on autoimmune disease.

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Development of X- and Y-cell retinogeniculate terminations in kittens

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The cat retinogeniculocortical pathways are organized chiefly into two parallel independent neuronal streams, one involving X-cells of the retina and lateral geniculate nucleus, and the other, Y-cells[1,2]. Development of the Y-cell pathway is more seriously affected by visual deprivation than is the X-cell pathway[2,3] and we reasoned that some insight into the underlying mechanisms of these effects could be gained from studies of normal development. We therefore injected horseradish peroxidase into physiologically identified X- and Y-cell retinogeniculate axons to examine the postnatal development of their terminations in kittens. As we report here, at 3–4 weeks of age, most optic tract axons can be identified physiologically as members of the X- or Y-cell class. X-cell terminal fields in lamina A or A1 are wider at 3–4 weeks than they are in adults, while Y-cell terminal fields are narrower than in adults[4,5]. During the second and third postnatal months, X-cell terminal arbors progressively contract while Y-cell arbors expand so that, by 12 weeks of age, the adult pattern is seen. These data, and the results of our earlier study of the effects of monocular lid suture on these terminal arbors[3], suggest that enlargement of Y-cell terminations in geniculate lamina A or A1 during development may be accompanied by competitive pruning of X-cell terminations within these same laminae.

Experiments were performed on three groups of kittens: 17 at 21–30 days old (the 3–4-week old group), 5 at 51–62 days old (the 8-week old group) and 5 at 81–91 days old (the 12-week old group). Animals in the 3–4- and 8-week old groups were anaesthetized with 1–2% halothane and paralysed with gallamine triethiodide; a respirator was used to hyperventilate them slightly with a mixture of 70% nitrous oxide, 29% oxygen and 1% carbon dioxide[6,7]. Our physiological and morphological methods for the 12-week old kittens were the same as those described previously for adult cats[4,8]. Briefly, micropipettes containing horseradish peroxidase (HRP) were used to record retinal fibres in the lateral geniculate nucleus (LGN) or in the subjacent optic tract. Each axon was identified as an X- or Y-cell on a battery of tests, including receptive field size, linearity of spatial and temporal summation, and axonal conduction velocity[9,10] (the application of these tests to kittens is described more fully below). The axon was then impaled and injected with HRP. A total of 190 retinal axons were recorded in these kittens and

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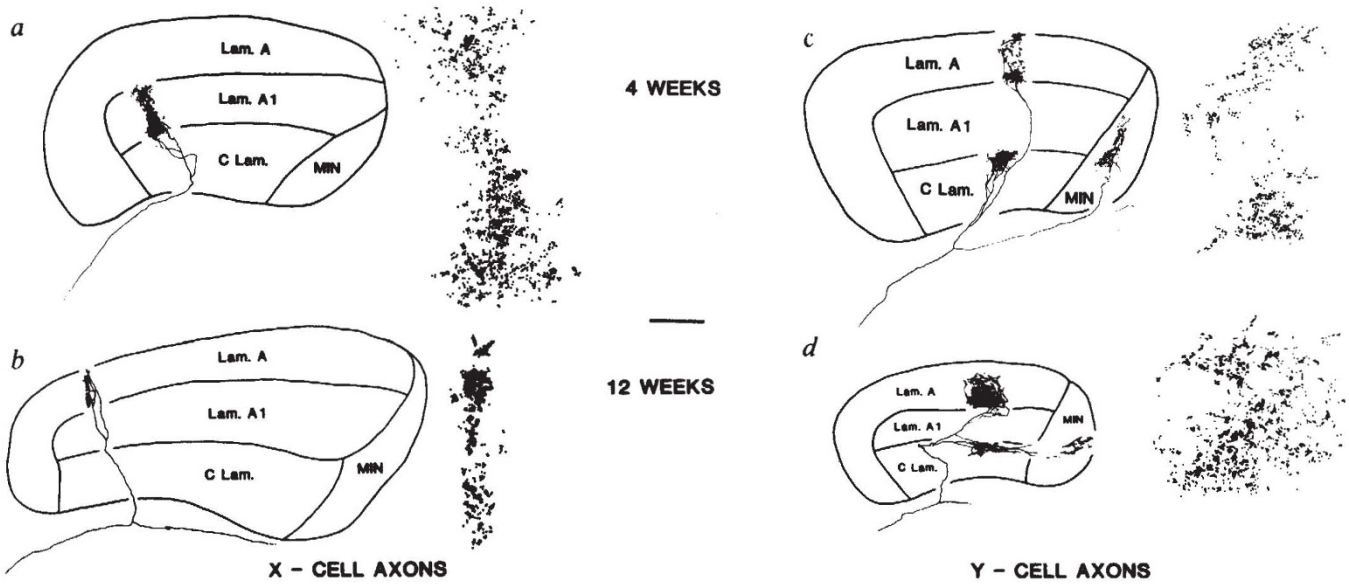

Fig. 1 Retinogeniculate arbors from single axons in kittens. Each axon is illustrated by a pair of drawings: left, a lower-power drawing of the arbor in the LGN; right, a higher-power drawing limited to the terminal boutons in lamina A or A1. The scale bar in the centre of the figure applies to all pairs (*a–d*) and represents 500 μm for the lower-power drawings and 100 μm for the higher-power ones. *a*, X-cell axon from the ipsilateral retina in a 30-day old kitten. The cell has an on-centre receptive field 2.0° in diameter located 26° from the vertical meridian and 38° below the horizontal zero parallel. Its latency to optic chiasm stimulation was 1.6 ms. Its responses to a counterphased, sine-wave grating exhibited linear spatial and temporal summation. This axon had 1,073 boutons in lamina A1. For comparison, X-cell axons from normal adult cats similarly analysed had 526–915 boutons in lamina A or A1[4]. *b*, Y-cell axon from the contralateral retina in a 23-day old kitten. The cell had an on-centre receptive field 3.3° in diameter located 5° from the vertical meridian and 6° below the horizontal zero parallel. Its latency to optic chiasm stimulation was 1.1 ms and its responses to a counterphased, sine-wave grating exhibited nonlinear summation. The axon had 565 boutons in lamina A, whereas 4 Y-cell axons from normal, adult cats similarly analysed had 651–1,405 boutons in lamina A or A1[4]. *c*, X-cell axon from the contralateral retina in an 81-day old kitten. The cell had an on-centre receptive field 0.9° in diameter located 35° from the vertical meridian and 1° below the horizontal zero parallel. Its latency to optic chiasm stimulation was 1.2 ms and it responded linearly to gratings. The terminal field in lamina A had 573 boutons. *d*, Y-cell axon from the contralateral retina in an 86-day old kitten. The cell had an off-centre receptive field 2.0° in diameter located 5° from the vertical meridian and 21° below the horizontal zero parallel. Its latency to optic chiasm stimulation was 0.6 ms and it responded nonlinearly to gratings. The terminal field in lamina A had 1,170 boutons.

of these, 65 were successfully filled with HRP and morphologically analysed. Brains were sectioned coronally or sagittally at 100 μm and reacted with 3-3-diaminobenzidine with cobalt intensification[11].

Physiologically, the 75 retinal axons recorded in 3–4-week old kittens could follow high frequencies (>100 Hz) of optic chiasm stimulation but had longer latencies to such stimulation than did axons in older kittens or adults. Although nonlinear responses to counterphased sine-wave gratings were sometimes variable in young kittens[7,12,13], these responses could nonetheless be reliably detected when 'null positions'[10] were used to minimize the linear response component. In the 3–4-week old kittens, the overwhelming majority of axons (46 of 49) identified as Y-cells had frequency-doubled, nonlinear response. Twenty-six axons identified as X-cells exhibited only linear responses. Y-cells exhibited a shorter latency to optic chiasm stimulation (mean 0.9 ms, range 0.6–1.2 ms) than did X-cells (mean 1.7 ms, range 1.2–2.5 ms). Receptive field centres for these cells in 3–4-week old kittens were roughly four times as large as in adults (mean for kitten X-cells 2.1°, range 1.0–5.0°; mean for kitten Y-cells 4.0°, range 1.7–7.5°; see ref. 12) and lacked detectable antagonistic surrounds. Y-cell receptive field centres were larger than X-cell centres at similar eccentricities. The three axons exhibiting only linear responses which were identified as Y-cells had chiasm latencies (0.9–1.1 ms) and field centres (3–4.5°) comparable with nonlinear Y-cells. Such 'linear' Y-cells have also been described in the kitten LGN[10]. None of these three was recovered histologically.

By 8 weeks of age, receptive field properties of the 54 recorded axons (29 X-cells and 25 Y-cells) were more adult-like than at 3–4 weeks of age. Compared with receptive fields of the younger kittens, those of 8-week old kittens were smaller and often comparable with adult sizes (see ref. 14), their surrounds were more developed and the nonlinear responses of the Y-cells were stronger and more stable. At 12 weeks of age, the 30 X-cells and 31 Y-cells were physiologically almost indistinguishable from those in adults[10,15].

Morphologically, X- and Y-cell terminations in 3–4-week old kittens clearly differed from those in adults both in the geometry of terminal zones in the A-laminae and in the sizes and shapes of individual terminal boutons. These differences were less pronounced at 8 weeks of age and by 12 weeks of age terminal fields appeared quite adult-like.

We successfully injected and recovered 10 X-cell and 12 Y-cell retinogeniculate axons in 3–4-week old kittens. Figure 1*a,c* illustrates representative X- and Y-cell terminal fields from these kittens. The X-cell axon arises from the ipsilateral retina, its terminal field is confined to lamina A1 of the LGN and it has a large number of boutons within a broad terminal field. The Y-cell axon from the contralateral retina innervates laminae C and A as well as the medial interlaminar nucleus of the LGN. Its terminal field in lamina A is quite narrow and sparse, with fewer boutons, compared with either X-cell arbors at similar ages or Y-cell terminal arbors in adults.

We have also recovered 12 X- and 7 Y-cell retinogeniculate axons from 8-week old kittens and 12 X- and 12 Y-cell axons from 12-week old kittens. Figure 1*b,d* illustrates representative arbors of an X- and a Y-cell from a 12-week old kitten. These arbors are not obviously different from those in adults[4], but they do differ from arbors in younger kittens. The X-cell terminal arbor in the A-laminae is much broader in the 3–4-week old kitten than in the 12-week old kitten, while the Y-cell arbor in the A-laminae is narrower and sparser in the 3–4-week old kitten than in the 12-week old kitten.

Figure 2 summarizes the postnatal development of terminal arbor widths in the A-laminae of X- and Y-cell retinogeniculate axons and also shows data from 16 X- and 12 Y-cell axons from adult cats[4]. X-cell axons gradually decrease in terminal arbor

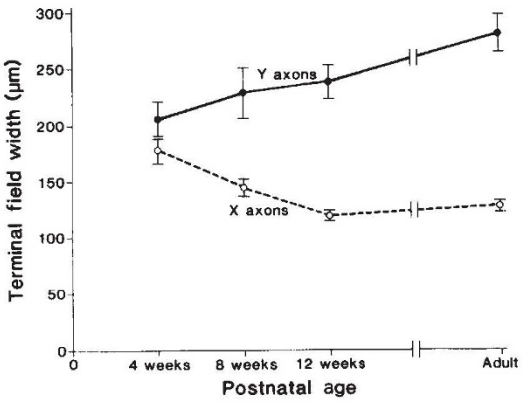

Fig. 2 Maximum width of axon terminal arbors (mean ± s.d.) in lamina A or A1 as a function of postnatal age.

width from 3–4 weeks of age until 12 weeks of age, while Y-cell axons increase in width to 12 weeks of age and beyond to adult values. Both trends are significant ($P < 0.01$ on a χ^2 test). Furthermore, the X- and Y-cell widths at 3–4 weeks are each significantly different from the corresponding values in 12-week old kittens or adults ($P < 0.05$ for the X-cell comparison between 3–4 and 12 weeks of age; $P < 0.01$ for the other comparison; Mann–Whitney U-test).

In addition to these postnatal changes in the geometry of terminal arbors, individual boutons also mature betwen 3 and 12 weeks postnatally[16,17]. Figure 3*a* illustrates a portion of the terminal field of an X-cell axon from a 27-day old kitten. Terminals at this age are often small and irregularly shaped compared with the well formed terminals seen at 12 weeks of age (Fig. 3*b*). Many terminals in the 3–4-week old kittens have spike-shaped or filopodial endings with growth-cone-like extensions (see ref. 17). Nonetheless, the clustering of terminals characteristic of X-cell arbors in 12-week old kittens (Fig. 3*b*) or in adult cats[4] already exists in the younger kittens studied (Fig. 3*a*). A Y-cell arbor in a 23-day old kitten (Fig. 3*c*, *d*) exhibits terminal morphology similar to that of the immature X-cell axon. In particular, Y-cell axons also exhibit numerous filopodial endings and terminations, and the swellings themselves appear generally smaller than those in 12-week old kittens (Fig. 3*e*) or adults[4].

The present results indicate that, during the critical period for visual development[18], there is dynamic shaping of the terminal boutons and terminal arbors of retinal X- and Y-cell axons in the LGN. Compared with retinal Y-cells, retinal X-cells seem to be generated earlier[19] and may innervate the LGN earlier[6,20]. Therefore, by a process analogous to that suggested for other developing systems[21], X-cell axons may initially have abundant terminations that retract or become pruned as Y-cells subsequently establish their arbors in the LGN, and the retraction of X-cell arbors and expansion of Y-cell arbors may occur by a process of mutual competition. Because much of the Y-cell expansion occurs during the critical period, eyelid suture during this time may interfere with the ability of Y-cell arbors to compete successfully for terminal space with the already developed X-cell arbors. Indeed, within the A-laminae, many X- and Y-cell arbors from the deprived eye of monocularly sutured cats exhibit geometry similar to that in 3–4-week old kittens and unlike that in adults[3]. It is also possible that the normal growth of Y-cell arbors and shrinkage of X-cell arbors are developmental processes independent of one another, that they involve no competitive interactions and that deprivation simply retards these developmental processes. However, Sur *et al.*[3] showed that retinogeniculate Y-cell axons from the deprived eye develop normally where X-cell arbors are never substantively present (that is, in lamina C) and that these Y-cell arbors fail to develop normally only in the presence of X-cell arbors (that is, in the A-laminae). This suggests that the development of retinogeniculate X- and Y-cell arbors is truly a competitive process.

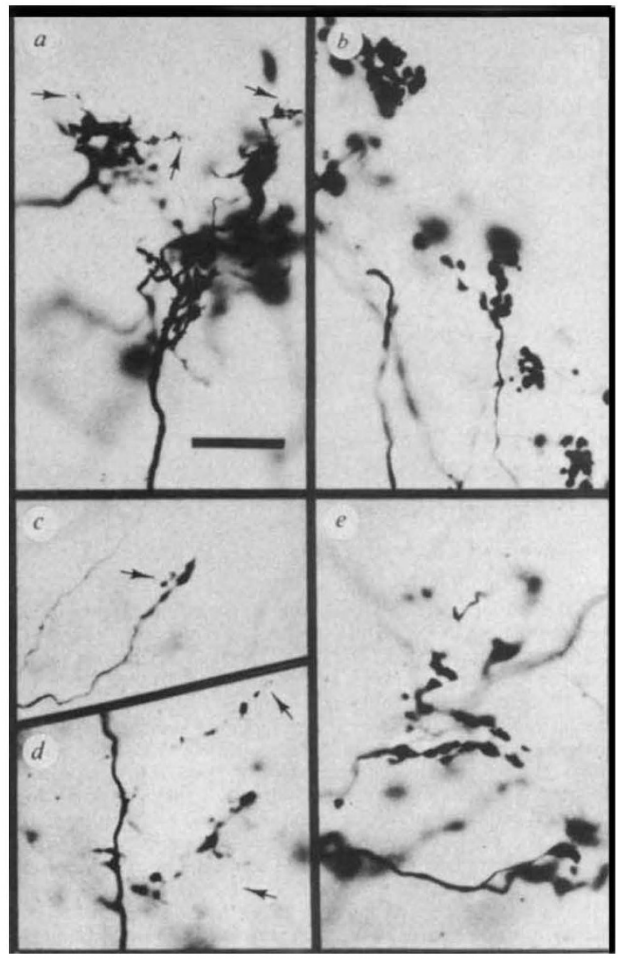

Fig. 3 Morphology of retinogeniculate terminal endings in kittens. *a*, Portion of X-cell terminal field in a 27-day old kitten. Arrows indicate filopodial or spike-shaped endings characteristic of terminals at this age. Scale bar, 25 μm, applying to *b*–*e* also. *b*, Portion of X-cell terminal field in a 91-day old kitten. Terminals have the form and features characteristic of adult X-cell axons. *c*, *d*, Portions of Y-cell terminal field in a 23-day old kitten. Arrows again indicate filopodial endings. Y-cell terminal fields are much sparser than X-cell arbors at this age. *e*, Portion of Y-cell terminal arbor in a 91-day old kitten showing adult-like terminal morphology.

Finally, we note that the pruning of X-cell arbors and growth of Y-cell arbors within individual lamina A or A1 probably occurs independently of the segregation of afferents from the two eyes within the LGN. The latter occurs prenatally[22] and does not seem to involve significant initial proliferation and later pruning of terminal fields[23]. Interactions between X- and Y-cell retinogeniculate terminations from the same eye appear to be superimposed postnatally on an afferent population that has already segregated into geniculate laminae.

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Expression of *myb*, *myc* and *fos* proto-oncogenes during the differentiation of a murine myeloid leukaemia

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It is widely thought that c-*onc* genes (or proto-oncogenes)—the cellular progenitors of retroviral transforming genes—are involved in cellular differentiation and/or proliferation. Such ideas originate primarily from the ability of v-*onc* genes and 'activated' c-*onc* genes to induce uncontrolled cellular proliferation, and their capacity to arrest or interfere with differentiation processes in some systems. Haematopoietic cell populations provide additional support for these ideas as c-*myb* RNA is present in cell lines corresponding to immature, but not mature, cell types[1,2], and elevated levels have been found in tissues that are active in haematopoiesis[2,3]. We have now examined the effects of induced differentiation on c-*onc* gene expression in a murine myeloid leukaemia cell line, WEHI-3B ('D$^+$' subline)[4]. Our results show that the expression of c-*myb* and c-*myc*, at the level of transcription, decreases only at late stages in the monocytic differentiation of WEHI-3B cells, while expression of c-*fos* increases markedly. We suggest that c-*myb* and c-*myc* do not themselves control myeloid differentiation, but that they function in the maintenance of the proliferative state of myeloid cells. The induction of c-*fos* may reflect its role in some macrophage-specific functions.

The cloned myelomonocytic leukaemia WEHI-3B can be induced to differentiate to both monocytes (macrophages) and granulocytes by granulocyte-colony stimulating factor[4] (G-CSF[5,6]). However, because G-CSF alone does not induce complete differentiation in cultures of a few days' duration, we have used G-CSF plus a low concentration of actinomycin D, a combination which Cooper *et al.*[7] have shown efficiently induces predominantly monocytic differentiation of WEHI-3B cells. In our hands, G-CSF plus actinomycin D routinely induced differentiation in ~90% of the WEHI-3B cells after 2 days' exposure. However, the degree of differentiation at this time varied between experiments, with differing proportions of promonocytes (Fig. 1*b*) and mature monocytes (Fig. 1*c*) being present (Table 1, expts 2–4). In all experiments, most cells were classified as mature monocytes by 3 to 4 days.

In order to examine c-*onc* expression in WEHI-3B cells, polyadenylated RNA was fractionated by formaldehyde–agarose gel electrophoresis and transferred to nitrocellulose filters. Hybridization to radiolabelled probes derived from cloned *onc* genes revealed the presence of transcripts corresponding to c-*abl*, c-Ki-*ras*, c-*fms*, c-*fes*, c-Ha-*ras*, c-*myb*, c-*myc* and c-*fos*. The c-*abl* and c-Ki-*ras* transcripts (Fig. 2) were of similar sizes to those reported elsewhere (refs 8 and 9, respectively). WEHI-3B cells contain two c-*fms* transcripts of 4.1 and 8.4 kilobases (kb) (Fig. 2), the smaller of which probably corresponds to the 3.7-kb transcript seen in mouse placental tissue[10]. The c-*fes* gene is also transcribed in WEHI-3B, giving rise to a single 3.2-kb RNA species. Although the size of the c-*fes* transcript has not previously been reported, we note that the avian homologue of c-*fes* (c-*fps*) is transcribed[11] and its product expressed[12] in haematopoietic cells. Transcription of c-*fes* and c-*fms* is under investigation and will not be dealt with further here. No transcripts of c-*src*, c-*sis*, c-*mos*, c-*erb-A* or c-*erb* were detected.

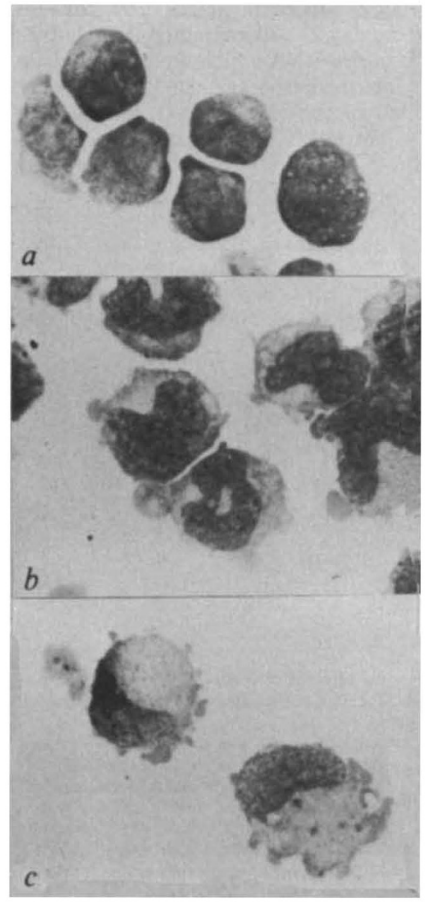

Fig. 1 Morphology of WEHI-3B cells undergoing monocytic differentiation. *a*, Undifferentiated WEHI-3B cells; *b*, promonocytes; *c*, monocytes appearing after induction of differentiation. **Methods:** WEHI-3B cells (D$^+$ subline)[4] were grown as described previously and treated with 370 U ml^{-1} G-CSF (partially purified by gel filtration from post-endotoxin serum)[5,6] plus 5 ng ml^{-1} actinomycin D. Cytocentrifuge preparations were stained with May–Grünwald/Giemsa.

Marked changes were observed in the levels of c-*myb*, c-*myc* and c-*fos* RNAs following induction of differentiation. For example, expt 1 (Table 1, Fig. 3) shows that differentiation of WEHI-3B cells to monocytes after 3 days of treatment with G-CSF plus actinomycin D resulted in a substantial decrease in the levels of c-*myb* and c-*myc* (6-fold and 10-fold, respectively, determined by optical densitometry of the autoradiograms), while transcription of c-*fos* increased (11-fold) and c-Ha-*ras* transcription was essentially unaltered.

To further correlate these changes in c-*onc* gene expression with different stages of WEHI-3B cell maturation, cells were collected after varying periods of induction, and independent experiments were compared in which the time course of differentiation had varied (Table 1, Fig. 3, expts 2–4). In expt 2, in which induction of differentiation for 2 days resulted in the differentiation of most of the cells to promonocytes, no significant changes in c-*myb* or c-*myc* RNA levels were observed. Furthermore, in expt 3, where most of the cells were classified as mature monocytes after 2 days' treatment (confirmed by staining cells for nonspecific esterase activity[13] and by monitoring the suppression of clonogenicity in soft agar cultures[4]), only a slight decrease in the levels of c-*myb* and c-*myc* was detected. This experiment shows that cells which had only just reached