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The Making of "Erytroid Islands" in Hema Culture

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In the marrow, macrophages form a morphological unit, the erythroblast island and in culture release factors still to be marked that affect the growth of the erythroblasts(Ery).When blood $CD34^{pos}$ are stimulated with dexamethasone(Dex) and growth factors in human erythroid massive amplification(HEMA)cultures, large numbers of Ery are generated which remain immature and proliferate for 10-17 days. Dex withdrawl and erythropoietin(EPO)exposure induce Ery to activate the maturation program within 24-48 h.At day 13-14 of HEMA. The role of macrophages in HEMA has not been investigated. Live contrast-phase microscopy(10x magnify.;time-lapses:1 frame/30"recorded for 8-10h)was used to visualize interactions between Ery and macrophages in day 13-14 HEMA with or without Dex and in cultures in which day 13-14 progeny were exposed to EPO alone. Macrophages and Ery at different stages of maturation were recognized on the basis of size, chromatin condensation state, motility and frequency using data previously obtained by FACS as comparison.Day14 without Dex: Ery at all maturation stages were observed moving in a coordinate fashion but with frequent changes in directions. Ery formed few, loose and unstable aggregates. Macrophages were rare and moved independently from Ery.Day14 with Dex: Ery moved synchronously toward each other forming large and stable aggregates. Macrophages were "fat", highly motile and extended short protrusions which established transient contacts with hundreds/thousands of Ery over 1 h. Contacts involved both isolated Ery (interactions, "loose")or clusters of Ery at all maturation stages(interactions "tight"). In the first 45'of observation,~45% of the interactions were loose,~23% were a combination of loose and tight and~35%were tight. By 1-2h,25% were loose,50% were loose and tight and 25% were tight. Cytokinesis involved single and double proEry. The process lasted 5' and was not associated with macrophages. Dex withdrawal and EPO exposure induced noticeable changes.By15',macrophages became greatly motile engaging in fewer "loose" interactions(5-9%)and in more "tight" interactions. By 60',86%of the interactions were "tight" and involved preferentially clusters of mature Ery (>80%).Mega-aggregates including 2-6 macrophage-Ery clusters each were also observed.