Proc. Natl. Acad. Sci. USA
Vol. 78, No. 1, pp. 323–327, January 1981
Cell Biology

The persisting (P) cell: Histamine content, regulation by a T cell-derived factor, origin from a bone marrow precursor, and relationship to mast cells

(P-cell stimulating factor/T-cell hybridoma/T-cell growth factor/multipotential hemopoietic stem cell/long-term bone marrow cultures)

JOHN W. SCHRADER*, STEPHEN J. LEWIS†, IAN CLARK-LEWIS*, AND JANETTA G. CULVENOR*

*The Walter and Eliza Hall Institute of Medical Research, Post Office Royal Melbourne Hospital, Victoria, 3050, Australia; and †Department of Pharmacology, University of Melbourne, Parkville, 3050, Australia

Communicated by Sir Macfarlane Burnet, September 22, 1980

ABSTRACT **Histamine was detected at levels of 100 ng/10^6 cells in the metachromatic granules of the persisting (P) cell, which appears in cultures of murine lymphoid or bone marrow cells and is capable of long-term growth *in vitro* in the presence of a T cell-derived growth factor. This factor, which we termed P-cell stimulating factor, was distinct from T-cell growth factor and had an apparent molecular weight of 25,000–30,000. P cells did not originate from Thy.1-positive cells nor was the thymus necessary for the development of their precursors. Moreover, P cells grew directly from colonies generated in agar cultures of bone marrow cells, the nature of the colonies indicating that P cells shared a common precursor with hemopoietic cells. Mutant W^f/W^f mice, although deficient in certain mast cells, possessed P-cell precursors. It is hypothesized that P cells are related to a specialized subset of mast cells, derived from a bone marrow progenitor but regulated by activated T cells.**

Two classes of cells are characterized by metachromatically staining cytoplasmic granules containing, among other substances, histamine. These cells, mast cells and basophils, act as effector cells in antibody-mediated (1) and T cell-mediated (2) hypersensitivity reactions. Burnet has suggested that at least some mast cells are postmitotic derivatives of T lymphocytes (3, 4), basing this proposal on his observation of the large numbers of mast cells in the thymus of certain mice (4) and, subsequently, upon observations including the growth of mast cells in tissue cultures of thymus cells (5, 6) and the failure of congenitally athymic (*nu/nu*) mice to respond to intestinal parasites with a local mastocytosis (7). Furthermore, it has been claimed that T cells are the precursors of the mast cells in the gut mucosa (8).

Here we demonstrate that cells that are capable of prolonged clonal growth *in vitro* in the presence of a T cell-derived factor and which we have termed persisting (P) cells (9, 10) contain histamine localized in large granules that resemble those of mast cells. These mast cell-like cells are generated from a bone marrow-derived progenitor common to other hemopoietic cells and not from T cells, but are regulated by a T cell-derived factor.

MATERIALS AND METHODS

Cell Culture. P cells were generated by culturing murine spleen cells, depleted of T cells with a monoclonal anti-Thy.1 antibody and complement, for 4 weeks at 10^5 cells per ml in Dulbecco's modified Eagle's medium supplemented with 2-mercaptoethanol (50 μM), asparagine (0.1 mM), glutamine (2.8 mM), and fetal calf serum (10%, vol/vol), together with medium conditioned by concanavalin A-stimulated spleen cells (CAS) (3%, vol/vol). For colony growth, medium was supplemented with 0.3% Bacto-Agar (Difco) and CAS (10%). Long-term bone marrow cultures (11) were set up as described (12), but with fetal calf, not horse, serum.

Preparation and Assay of Growth Factors. CAS was prepared by concentration (10×) of supernatants of 24-hr cultures of CBA spleen cells (5×10^6/ml) in modified Eagle's medium supplemented with 2.5 μg of concanavalin A per ml (Pharmacia). Supernatants of T-cell hybridoma 123 were prepared as described (13). T-cell growth factor (TCGF) or P-cell stimulating factor (PSF) was assayed by adding 10 μl of either concanavalin A-activated T-cell blasts (2×10^4/ml) or P cells (5×10^3/ml) to 2-μl test samples in the wells of a Terasaki plate and scoring T- or P-cell growth visually at day 3 or 7, respectively.

Histamine Assays. Cells were suspended in 0.1 ml of phosphate-buffered saline and boiled for 10 min. Histamine was isolated and assayed spectrophotofluorometrically (14). The procedure was highly selective and sensitive for histamine. Large concentrations of L-histidine, spermidine, serotonin, carnosine, histidyl-L-histidine, and arginine produced negligible fluorescence, whereas 1 ng of histamine per ml gave levels of fluorescence twice that of the blank.

RESULTS

Previously we described the P cell as a cell that grew *in vitro* for periods exceeding 100 days when spleen, thymus, lymph node, or bone marrow cells from a variety of mouse strains were cultured with CAS (9, 10). P cells grew only if CAS were present, had a doubling time of approximately 36 hr, and could be cloned by agar or limit-dilution techniques. The extensive proliferative capacity of P-cell cultures in which all cells had the typical granulated morphology described below, the observation of mitoses in granulated cells, and the relatively high cloning efficiency of P cells in liquid or agar (10%) all suggested that P cells were self-renewing cells. P cells lacked lymphoid markers, being negative for Ig, Thy.1, Lyt.1, and Lyt.2 antigens, and were nonadherent and nonphagocytic, but had Fc receptors and H-2 and Ia antigens (9, 10).

A striking feature of P cells was the presence of coarse cytoplasmic granules that were water soluble and stained metachromatically with Toluidine blue; these are also characteristics of mast cell granules, as first described by Ehrlich (15). Astra blue, which stains mucopolysaccharides and has a specific affinity for mast cell granules (16, 17), stained the granules in P cells bright blue (Figs. 3–5).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: CAS, medium conditioned by concanavalin A-stimulated spleen cells; CFU-s, splenic colony-forming unit; CSF, colony-stimulating factor; P cell, persisting cell; PSF, P-cell stimulating factor; TCGF, T-cell growth factor.

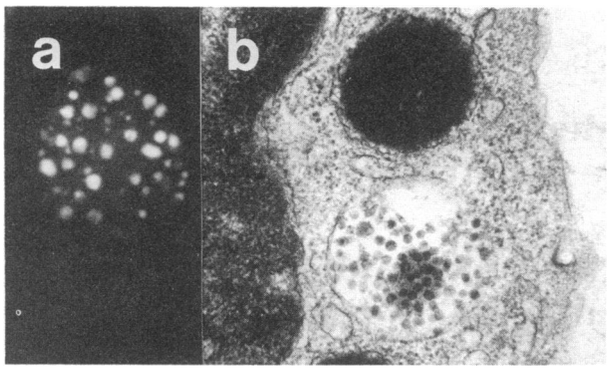

FIG. 1. (*a*) Fluorescence micrograph of a P cell cytocentrifuged and stained with *o*-phthaldialdehyde. (×2300.) (*b*) Detail of an electron micrograph of a P cell. (×25,600.)

P Cells Contain Histamine. We detected histamine in P cells, by using a sensitive and selective method (14), at levels of 24–210 ng/10^6 cells. The level of histamine measured by this technique in a population of resident peritoneal cells from (C57Bl/6 × DBA/2)F_1 hybrid mice was 330 ng/10^6 cells, corresponding to a level of 7 μg/10^6 of the mast cells present.

P-Cell Histamine Is Localized Within Granules. The characteristic granules of mast cells and basophils are storage sites for histamine. To determine whether P-cell granules also contained histamine, we used *o*-phthaldialdehyde to identify and localize histamine within P cells by fluorescence (18). As shown in Fig. 1*a*, the P-cell granules fluoresced brightly. Control cells (T-cell hybridoma 123 and nonmast cells present in resident peritoneal cell populations) had only a pale blue fluorescence, whereas mast-cell granules fluoresced bright yellow.

Electron Microscopy. The most prominent features in electron micrographs of P cells were the large electron-dense granules, resembling those of mast cells (Fig. 1*b*). In some cases, the electron-dense material appeared to be made up of an aggregation of smaller granules (Fig. 1*b*). Sometimes almost empty vacuoles containing only a few of the smaller granules were seen.

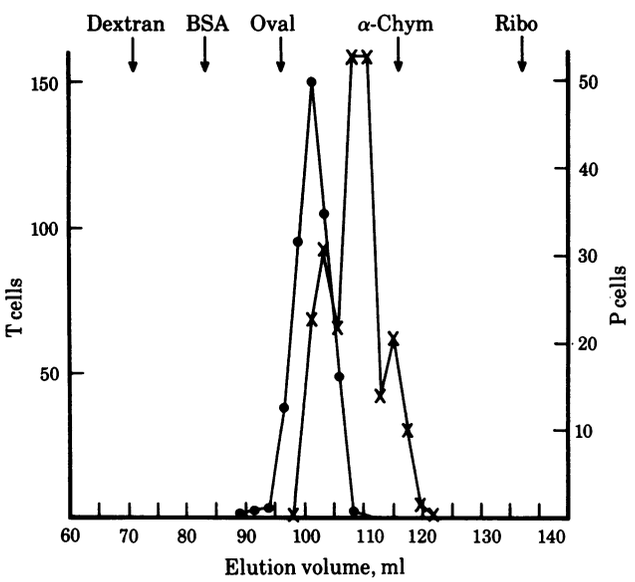

FIG. 2. Gel filtration on Sephadex G-75 of medium conditioned by concanavalin A-stimulated T-cell hybridoma 123 cells. PSF and TCGF were assayed as described. The molecular weight markers were bovine serum albumin (BSA), ovalbumin (Ova), α-chymotrypsinogen (α-Chym), and ribonuclease (Ribo). ●, T cells; ×, P cells.

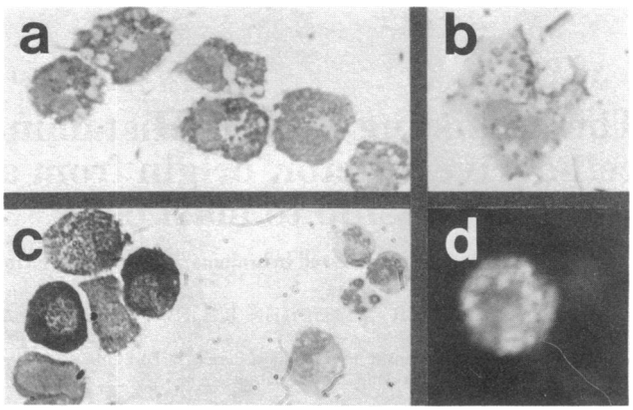

FIG. 3. (*a*) Cytocentrifuge preparation of P cells grown from bone marrow that had been treated with anti-Thy.1 monoclonal antibodies plus complement. (May–Grünwald–Giemsa stain; ×1200.) (*b–d*) Cells from long-term (8-week) cultures of (CBA × BALB/c)F_1 hybrid bone marrow. (*b*: Astra blue, ×1200; *c*: May–Grünwald–Giemsa stain, ×1200; *d*: *o*-phthaldialdehyde, ×1500.)

A Specific T Cell-Derived Factor Supports P-Cell Growth. Medium conditioned by concanavalin A-stimulated T-cell hybridoma 123 supported P-cell growth (9, 10), indicating that the relevant factor was produced by the T cell. Because both this T-cell hybridoma-conditioned medium (9) and CAS contain TCGF (19) and there is a suggestion that mast cells might arise from T cells, it was important to determine whether TCGF was the factor required for P-cell growth. Gel filtration experiments established that the T-cell and P-cell growth-supporting activities were due to different molecules: the apparent molecular weight of the TCGF produced by hybridoma 123 was 35,000–40,000, whereas that of the factor stimulating P-cell growth (PSF) was 25,000–30,000 (Fig. 2).

P-Cell Precursors Are Not T Cells. Astra blue-positive cells resembling P cells were not detected in smears of spleen, thymus, or bone marrow cells, all sources of P cells, suggesting that P cells generated *in vitro* were derived from a precursor cell. One possibility was that this precursor was a T cell, but two pieces of evidence suggested that this was not so. First, P cells could be grown from spleen or bone marrow cells that had been treated with anti-Thy.1 antibody plus complement to remove T cells (Fig. 3*a*). Second, P cells could be grown from cultures of bone marrow from congenitally athymic (*nu/nu*) mice, showing that the presence of the thymus was not necessary for the development of P-cell precursors.

Lack of Obligatory Role for T Cells in P-Cell Production. Long-term bone marrow cultures (11) contained cells resembling P cells or mast cells; they had cytoplasmic granules that stained bright blue with Astra blue (Fig. 3*b*) and darkly with May–Grünwald–Giemsa stain (Fig. 3*c*) and fluoresced bright yellow with *o*-phthaldialdehyde (Fig. 3*d*). The frequency of such cells was about 1%, but rose rapidly when nonadherent cells from long-term bone marrow cultures were subcultured with CAS. By 3 weeks, an apparently homogeneous population of continuously growing P cells had developed. Although long-term bone marrow cultures contain small numbers of Thy.1-positive cells [<1% (9)], there was no deliberate addition of T-cell mitogens to the system and, thus, we would not expect the production of PSF. Indeed, medium conditioned by these long-term bone marrow cultures was not able to support P-cell growth or survival. Thus, it appears that PSF is not required for the *in vitro* survival and production of P cells under all conditions, but acts rather as a regulator of P-cell growth.

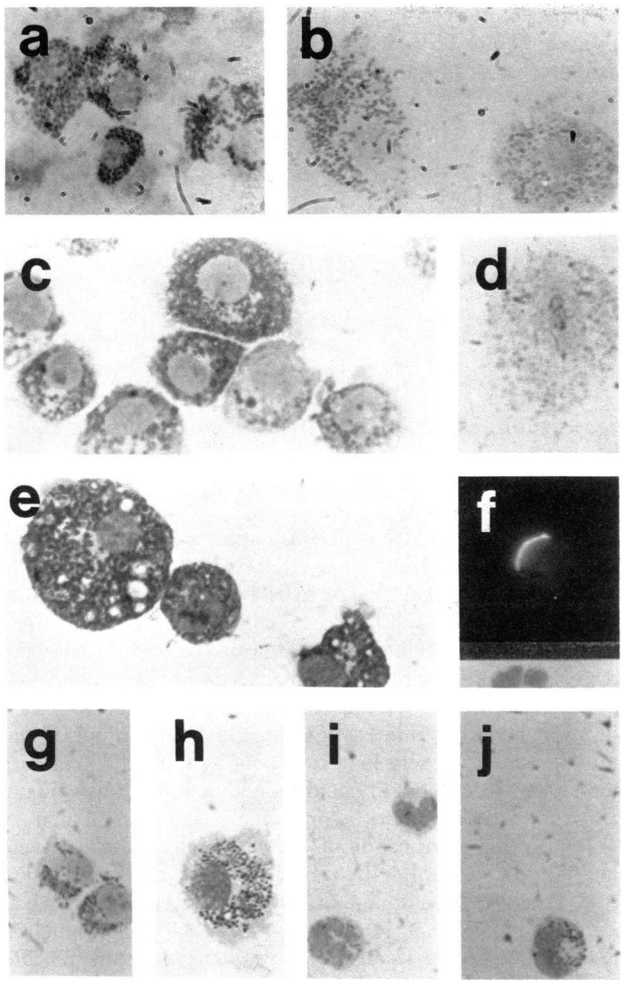

FIG. 4. (*a* and *b*) Astra blue-positive cells from agar colonies. (*c–e*) P cells from cultures derived from three individual agar colonies and stained with May–Grünwald–Giemsa stain (*c* and *e*) or Astra blue (*d*). (*f*) Colony-derived P cell stained by indirect immunofluorescence with ATH anti-ATL antiserum. (*g* and *i*) Smears of cells in two 13-day colonies stained with May–Grünwald–Giemsa stain. (*h* and *j*) Cells grown from the corresponding colonies (see *g* and *i*) at day 20. (×1200.)

P Cells Can Be Directly Derived from Myeloid Cell-Containing Bone Marrow Colonies in Agar. We, like others (17), did not detect Astra blue-positive cells in bone marrow. However, when bone marrow cells were cultured with CAS, P cells appeared, forming 2% of the population after 4 days and more than 95% after 3 weeks, although splenic colony-forming units (CFU-s) (20) persisted for at least 4 weeks. P cells continued to grow for 3 months or more. To identify the precursor of these P cells and to determine whether this cell also gave rise to other cell types, we cultured bone marrow cells with CAS in agar so that cell growth occurred in colonies.

When CBA bone marrow was cultured in agar with CAS, at day 7 about 60% of the colonies contained granulocytes or macrophages (or both) and up to 40% contained megakaryocytes. However, many cells, particularly in the largest colonies, were relatively undifferentiated; possibly these had differentiative capacities that were not fully expressed under the culture conditions used. At 2 weeks, a high percentage of these colonies (15 out of 25; 40 out of 56) contained cells resembling P cells (Fig. 4 *a*, *b*, and *g*). The granules in these cells stained brightly with Astra blue (Fig. 4 *a*, and *b*), like those of P cells or mast cells. Although macrophages appeared to be readily distinguishable from these cells (either not staining or having smaller granules that stained only weakly), it is unwise to identify cells grown in a polysaccharide matrix of agar on the basis of cytoplasmic structures containing polysaccharides.

Table 1. Generation of cultures of P cells from individual colonies of bone marrow cells in agar

Agar colonies*			No. of subcultures with P cells at succeeding weeks						
Group	Day	*n*	3	5	6	7	8	9	10
1†	17	20		20	10	3	1	0	
2	11	9	9	8		2		1	1
3	9	48	12		4	2	2		
4	13	20	3	3	1				
5	13	9	5	5	4				
6	13	7	5	3					
7	13	23	6	6	5				

* Bone marrow cells (10^5) from normal CBA mice, mice treated 1 day before with 5-fluorouracil (5 mg intravenously) (group 5), or normal (C57Bl/6 × DBA/2)F_1 hybrid mice (group 7) were cultured in agar with CAS or supernatant of hybridoma 123 (group 6). Groups of the indicated numbers (*n*) of individual colonies were transferred on the day shown to 0.2-ml liquid cultures. Each week, growing cells were passaged, smeared, and stained to identify P cells.

† Microbial contamination in group 1 ended six cultures during week 6 and one culture during week 9.

Therefore, individual large colonies were transferred to liquid cultures (0.2 ml) containing medium plus CAS. At weekly intervals, the liquid cultures were assessed for growth and the cells were passaged in 1- to 10-ml cultures, depending on the growth rate of individual cultures. The dominant cells in cytocentrifuged samples of the growing cultures were P cells. In a minority of early cultures (eight of those in groups 4–7, Table 1), 10% or less of the cells were polymorphs, but these did not persist beyond 4 weeks. Sometimes early cultures contained, in addition to P cells, unidentifiable, undifferentiated cells which did not persist. The P cells derived from bone marrow colonies had a typical appearance (Fig. 4 *c–e*), and the granules stained with Toluidine blue, Astra blue (Fig. 4*d*), and *o*-phthaldialdehyde. Cells derived from a culture of a CBA bone marrow colony bound ATH anti-ATL antiserum (Fig. 4*f*), indicating the presence of Ia antigens, as detected on P cells derived from spleen.

As shown in Table 1, P cells grew from a significant but variable proportion of the colonies, the variability perhaps reflecting differences in the periods of culture in agar and in the conditioned media used. The P cells derived from agar colonies showed considerable heterogeneity in their capacity for continued growth; the total number of P cells produced per colony ranged from thousands to millions. Only a minority of the cultures grew for more than a month, but three cultures have been monitored for more than 10 weeks and a fourth culture was lost through contamination during week 9. Further experience is needed before a reliable estimate of the self-renewal capacity of these cultures can be made.

Given that more than 90% of the colonies at day 7 contain cells classifiable as granulocytes, macrophages, or megakaryocytes, these results suggest that the P cells were generated from a cell with the capacity to produce other cell types. In four of the groups shown in Table 1 (groups 4–7), only half of the colony was transferred to a secondary culture; the other half was cytocentrifuged and stained with May–Grünwald–Giemsa stain. In a large proportion of these day 13 colonies (47%, 78%, 100%, and 90% in groups 4–7; Table 1), 50–100% of the cells resembled P cells (Fig. 4*g*); the remainder were blasts or immature myeloid cells. The other colonies contained megakary-

Table 2. Cultures derived from single agar colonies may contain both P cells and CFU-s

Exp.*	Day	Cell dose	Mean CFU-s/mouse	
			Controls†	Injected
1	38	5×10^4	0	5.5, 0.6, 3.7, 0
2	17	10^3–10^4	0.14	1, 2, 0.5, 1.5, 1, 0, 1
2	31	10^3–10^4		2, 0, 1.5, 0.5, 0

* Cells from selected rapidly growing cultures containing 5×10^4–5×10^5 cells were injected at the indicated day into two to four lethally irradiated (850 rad) syngeneic mice and CFU-s were counted at day 9. Four cultures were tested in Exp. 1, and a total of 12 in two groups in Exp. 2.

† Background colonies were determined in four and seven control, uninjected, irradiated mice in Exps. 1 and 2, respectively.

ocytes or, rarely, macrophages, and these did not appear to generate growing cells in the secondary cultures. Polymorphs were detected at day 21 among P cells in eight of the cultures in groups 5 and 6 (Fig. 4*j*). The multipotential nature of some of the colony-forming cells that ultimately gave rise to P cells was most strikingly shown in experiments in which we injected cells from the most rapidly growing cultures into irradiated mice and detected a low frequency (0.1–$1/10^3$ cells) of CFU-s (Table 2).

Relationship of P Cells to Mast Cells. The extensive self-renewal capacity of the P cell suggested that it might be a precursor of mast cells. In this respect, the *W* mutation is of interest because W/W^v mice are grossly deficient in mast cells, a deficiency that can be rectified by a bone marrow graft (21). Other hematological defects in *W/W* mice seem to reflect deficiencies in stem cells (22) or in a Thy.1-positive cell (23), and the lack of mast cells in the W/W^v mouse may reflect a deficiency in either a precursor cell or a regulatory molecule. We did not detect mast cells in the peritoneal cavity or mesentery of (C57Bl/6 × DBA/2)F_1 hybrid W^f/W^f mice. However, typical P cells, with the characteristic *in vitro* growth properties, histamine content, and cytological features, were generated from W^f/W^f spleen or bone marrow (Fig. 5). The normal kinetics of appearance of P cells in W^f/W^f bone marrow cultures suggested that there was no quantitative deficiency in P-cell precursors in W^f/W^f bone marrow, as is the case with committed granulocyte/macrophage progenitor cells (24). Furthermore, W^f/W^f T cells were able to generate PSF. It is possible that W^f/W^f mice have a qualitative or quantitative defect in P-cell precursors that was not apparent in our experiments; alternatively, the P cell may not be directly related to those mast cells absent in W^f/W^f mice.

DISCUSSION

Histamine. The amounts of histamine in P cells were ≈100 ng/10^6 cells and were less than those estimated for peritoneal mast cells (7 μg/10^6 cells). This difference is consistent with the difference in the number of granules in P cells and peritoneal mast cells. There is, however, considerable heterogeneity in the degree of granulation in cultures of P cells, and even in cloned lines a significant proportion of P cells contain so many granules that they closely resemble mast cells. The presence of feeder layers (7) and activated T cells (6) appears to increase the histamine content of cultured mast cells.

The important feature of P cells is that they appear to store histamine in specialized granules that have the same affinity for dyes such as Toluidine blue or Astra blue as have the granules of mast cells or basophils. Presumably, the granules contain similar acid mucopolysaccharides. Various cells may make, but not store, histamine (25). The bone marrow has very high levels of the histamine-generating enzyme histidine decarboxylase (25). Cytochemical studies suggest that histamine occurs in immature granulocytic cells in normal bone marrow and in mature granulocytes in chronic myeloid leukemia (26). However, definitive conclusions about the presence of histamine in a given cell require the purification of that cell and the isolation and quantitation of histamine by a specific assay, as reported here for the P cell. It is possible that histamine-storing cells such as P cells may have some role in growth regulation inasmuch as CFU-s (27) and some T cells have histamine receptors (28, 29).

PSF and Regulation of P Cells. We have shown that the T cell-derived PSF has an apparent molecular weight of 25,000–30,000 and is distinct from TCGF (Fig. 2). PSF has been completely separated from TCGF by hydrophobic chromatography (not shown). With regard to the relationship of PSF to the T cell-derived CSFs (30), at least one T cell-derived CSF

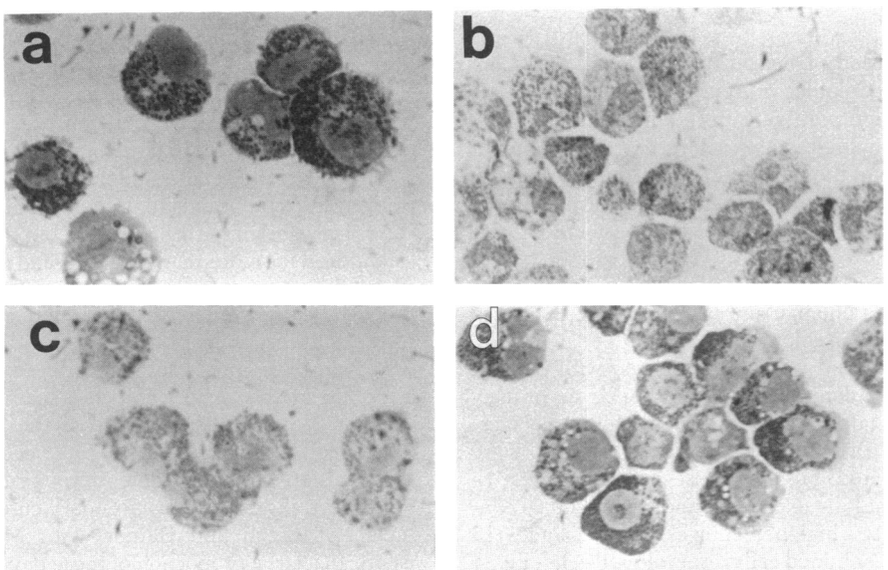

FIG. 5. (*a–c*) P cells, derived from W^f/W^f mice, stained with May–Grünwald–Giemsa stain (*a*), Astra blue (*b*), and Toluidine blue (*c*). (*d*) P cells from control cultures of wild-type bone marrow stained with May–Grünwald–Giemsa stain. (×850.)

does not affect P cells inasmuch as another T-cell hybridoma, T19.1, produces a granulocyte/macrophage CSF but not PSF. Two non-T cell-derived CSFs, from mouse lung (30) or L cells (31), had no effect on P cells (ref. 32 and unpublished observations).

The presence of PSF was not obligatory for the production of histamine-storing cells; they were generated in the absence of PSF in long-term bone marrow cultures (Fig. 3 *b–d*) although, when cells from these long-term cultures were cultured with PSF, P-cell numbers rapidly increased. This situation, in which the T cell-derived PSF regulates but is apparently not essential for the production of P cells, parallels observations on erythrocyte and eosinophil production. Although regulated by T cell-dependent CSFs, erythrocyte and eosinophil production occurs at normal basal levels in congenitally athymic (*nu/nu*) mice (24, 30).

Origin of P Cells and Their Relationship to Mast Cells. P cells were not derived from thymus-processed Thy.1-positive cells (Fig. 3*a*), but could be generated from bone marrow precursors that, at least in some cases, were able to give rise also to polymorphs (Fig. 4*j*) and CFU-s (Table 2). Although peripheral to this report, the prolonged culture of the progeny of a single pluripotential stem cell, including CFU-s, is a significant technical advance and will be fully documented elsewhere.

Both biochemically and structurally, the P cell seems likely to be related to the mast cell, although it is important to note that mast cells are heterogeneous; for example, mast cells in connective tissue or serosa differ from those in gut mucosa (4, 33). Several groups have described the growth in cultures of cells from thymus or lymph nodes; these cells contain histamine (5, 6), have metachromatic granules (5, 6) and IgE receptors (6), and degranulate with agents such as compound 48/80 (5, 6). It is likely that these cells are identical to P cells, and that their growth has been stimulated by the release of PSF from T cells in the cultures. Electron micrographs of thymus-derived mast cells (5) show granules identical to those in Fig. 2. It has been reported that T cells in the gut mucosa have mast cell-like granules and can mature to mast cells (8), but it is intriguing to speculate that these granulated "lymphocytes" are instead relatives of the P cell. Interestingly, we have found granulated gut lymphocytes in W^f/W^f mutant mice.

The observation that W^f/W^f mice have both P-cell precursors (Fig. 5) and T cells capable of producing PSF weighs against the notion that P cells are the precursors of the skin and gut mast cells reported to be lacking in *W/W* mice (21). However, it will be important to determine whether *W/W* mice also fail to respond with an intestinal mastocytosis when challenged with an appropriate parasite. At present, it seems that P cells are related to at least those members of Burnet's MC1 category of mast cell (4) that have been derived from lymphoid tissues *in vitro* (5, 6); they are possibly also related to those mast cells involved in the T cell-dependent responses to intestinal parasites. Certainly, data on the P cell point to the existence of a widely distributed pool of precursors of histamine-storing cells, the expansion and differentiation of which is regulated by activated T cells.

We thank Prof. G. J. V. Nossal and Dr. D. Metcalf for helpful discussions and Ms. D. Grail and I. Frangeskakis for excellent technical assistance. This study was supported by the National Health and Medical Research Council (Canberra, Australia), The Bushell Trust, and the Windermere Foundation.

1. Ishizaka, T. & Ishizaka, K. (1975) *Prog. Allergy* **19**, 60–121.
2. Askenase, P. W. (1977) *Prog. Allergy* **23**, 199–320.
3. Burnet, F. M. (1965) *J. Pathol. Bacteriol.* **89**, 271–284.
4. Burnet, F. M. (1977) *Cell. Immunol.* **30**, 358–360.
5. Ginsburg, H., Hammel, I., Eren, R., Weissman, B. A. & Yehudith, N. (1978) *Immunology* **35**, 485–502.
6. Ishizaka, T., Adachi, T., Chang, T. & Ishizaka, K. (1977) *J. Immunol.* **118**, 211–217.
7. Ruitenberg, E. J. & Elgersma, A. (1976) *Nature (London)* **264**, 258–260.
8. Guy-Grand, D., Griscelli, C. & Vassali, P. (1978) *J. Exp. Med.* **148**, 1661–1677.
9. Schrader, J. W., Clark-Lewis, I. & Bartlett, P. (1980) in *ICN-UCLA Symposia on Molecular and Cellular Biology*, eds. Gale, R. P. & Fox, C. F. (Academic, New York), Vol. 17, pp. 443–459.
10. Schrader, J. W. & Nossal, G. J. V. (1980) *Immunol. Rev.* **53**, 60–84.
11. Dexter, T. M., Allen, T. D. & Lajtha, L. G. (1977) *J. Cell. Physiol.* **91**, 335–344.
12. Schrader, J. W. & Schrader, S. (1978) *J. Exp. Med.* **148**, 823–828.
13. Schrader, J. W., Arnold, B. & Clark-Lewis, I. (1980) *Nature (London)* **283**, 197–199.
14. Lewis, S. J., Fennessy, M. R., Laska, F. J. & Taylor, D. A. (1980) *Agents Actions* **10**, 197–205.
15. Ehrlich, P. (1879) *Arch. Anat. Physiol. (Leipzig)* **3**, 166–169.
16. Bloom, G. & Kelly, J. W. (1960) *Histochemistry* **2**, 48–57.
17. McCarthy, J. H., Mandel, T. E., Garson, O. M. & Metcalf, D. (1980) *Exp. Hematol.* **8**, 562–567.
18. Juhlin, L. & Shelley, W. B. (1966) *J. Histochem. Cytochem.* **14**, 525–528.
19. Gillis, S., Ferm, M. M., Ou, W. A. & Smith, K. A. (1978) *J. Immunol.* **120**, 2027–2032.
20. Till, J. E. & McCulloch, E. A. (1961) *Radiat. Res.* **14**, 213–222.
21. Kitamura, Y., Go, S. & Hatanaka, K. (1978) *Blood* **52**, 447–452.
22. McCulloch, E. A., Siminovitch, L. & Till, J. E. (1964) *Science* **144**, 844–846.
23. Wiktor-Jedrzejczak, W., Sharkis, S., Ahmed, A., Sell, K. W. & Santos, G. W. (1977) *Science* **196**, 313–315.
24. Metcalf, D. & Moore, M. A. S. (1971) *Hemopoietic Cells* (North-Holland, Amsterdam), pp. 493–495.
25. Kahlson, G. & Rosengren, E. (1971) *Biogenesis and Physiology of Histamine* (Arnold, London), pp. 217–251.
26. Catini, C., Gheri, G. & Miliani, A. (1977) *Histochem. J.* **9**, 141–151.
27. Byron, J. W. (1978) in *Differentiation of Normal and Neoplastic Hematopoietic Cells*, eds. Clarkson, B., Marks, P. A. & Till, J. E. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 121–130.
28. Plaut, M., Lichtenstein, L. M., Gillespie, E. & Henney, C. S. (1973) *J. Immunol.* **111**, 389–394.
29. Shearer, G. M., Weinstein, Y. & Melmon, K. L. (1974) *J. Immunol.* **113**, 597–607.
30. Metcalf, D. (1979) in *Biology of the Lymphokines*, eds. Cohen, S., Pick, E. & Oppenheim, J. (Academic, New York), pp. 515–540.
31. Stanley, E. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2969–2973.
32. Schrader, J. W. (1981) *J. Immunol.* **126**, in press.
33. Mayrhoffer, G., Bazin, H. & Gowans, J. L. (1976) *Eur. J. Immunol.* **6**, 537–545.