Lymphocytes and Hemic Stem Cells

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MUCH RECENT WORK on hematopoietic stem cells has involved the spleen colony technic and has been primarily concerned with those hemic precursors which ultimately differentiate into myeloid, erythroid and megakaryocytic elements. There has also been considerable investigation of the differentiation of cells with different immunologic functions within the lymphoid series, but relatively little is known of the interrelationships between lymphoid and hematopoietic elements.

It is the purpose of this presentation to review several studies, including some from our own laboratories, that are concerned with a common precursor of both hematopoietic and lymphoid cells, and then to consider briefly contributions of these studies to our understanding of differentiation within the immune system itself.

Evidence for a Pluripotential Lymphohematopoietic Stem Cell

The concept of a common precursor for all blood cells, including lymphocytes, is an old one, but the possibility that such stem cells persist into adult life has long been debated. One experimental approach to this problem was provided by the advent of modern mammalian cytogenetic technics, and in 1959, Barnes et al [1] demonstrated in several mice that had spontaneously recovered from sublethal whole-body irradiation, repopulation of lymphoid tissues as well as bone marrow by cells bearing the same radiation-induced chromosome markers. Thus, the thymus and lymph nodes as well as the spleen and bone marrow apparently contained differentiated cells, all descended from a common precursor.

Subsequently, Wu et al,[2] Trentin et al [3] and others, made additional studies in mice using various combinations of transplantation and cytogenetic technics. Wu et al,[2] with a two-stage marrow transplantation method, were able to repopulate irradiated mice with clones of cells derived from hematopoietic stem cells, and demonstrated that cells bear-

ing the same chromosome markers could repopulate thymus and lymph nodes as well as large areas of spleen and bone marrow. They inferred that the cells repopulating the lymph nodes were functionally competent, since the hosts were able to produce antibody after immunization with sheep red blood cells. The authors concluded that the "precursors of cells in thymus and lymph nodes are identical with hematopoietic colony-forming cells, or they are both descendents from a common precursor."

Trentin et al [3] extended these investigations by demonstrating that cells derived from hematopoietic spleen colonies, which themselves did not contain antibody-forming elements, could, when transplanted into secondary hosts, repopulate the lymphoid organs with antibody-forming cells. Again using radiation-induced chromosomal aberrations as markers, these studies indicated that undifferentiated stem cells contained in spleen colonies not only could produce erythroid, myeloid, and megakaryocytic elements, but could also be the precursors of functioning immunocompetent lymphocytes. In fact, in this investigation, cells of the same chromosomally marked clone were shown to be immunologically responsive to three different antigens.

Additional evidence that immunocompetent lymphocytes as well as marrow elements may be derived from a common stem cell resulted from recent studies with rats in our own laboratories.[4] Rats were sublethally irradiated and allowed to recover spontaneously to permit repopulation of the hematopoietic tissues with clones of cells marked by radiation-induced chromosome aberrations. Subsequently, bone marrow cells from rats with satisfactorily marked clones were injected into lethally irradiated mice to form hematopoietic spleen colonies, and peripheral blood lymphocytes from the same rats were stimulated to proliferate in mixed leukocyte cultures (MLI) or with phytohemagglutinin (PHA). In several instances, cells with the same radiation-induced chromosome markers constituted erythroid colonies in the mouse spleen and proliferated in the mixed lymphocyte cultures. As the MLI has been considered a valid model of at least the first stages of an immunologic response to major histocompatibility isoantigens,[5] these observations were considered to indicate the presence in the adult rat of a pluripotential lymphohematopoietic stem cell capable of yielding both erythroid and immunocompetent lymphoid progeny.

Taken together, these various experimental studies, each employing slightly different technics, strongly suggest that there are primitive stem cells in the adult rat and mouse that are capable of differentiating, at least under these very extreme experimental circumstances, into func-

tional progeny not only within the myeloid-erythroid-megakaryocytic complex, but as immunocompetent lymphocytes as well.
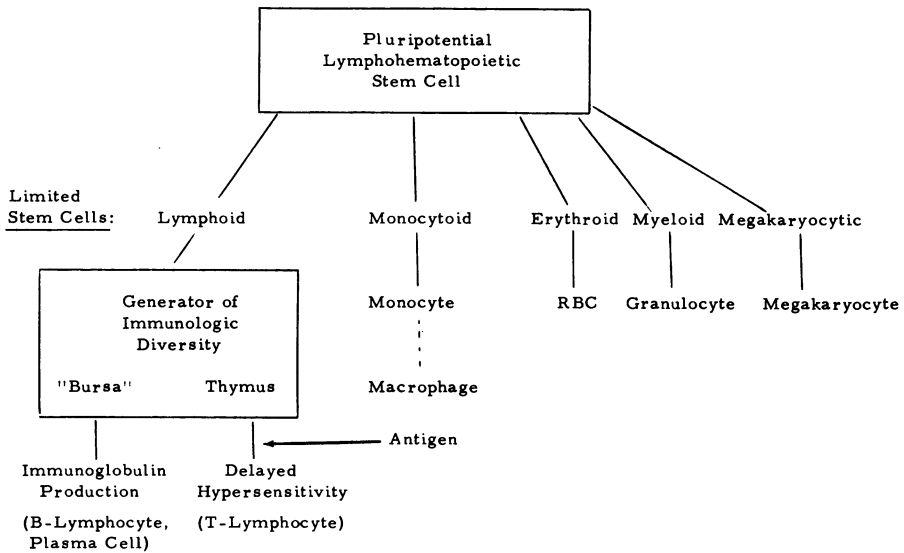
Characteristics of the Pluripotential Lymphohematopoietic Stem Cell

Very little is known about the number, distribution within the body or morphology of this multipotent hemic stem cell. It is apparent that it need not be present in very large numbers in the adult animal. In rats and mice after recovery from sublethal irradiation, the marked clones derived from these precursor cells have been observed to constitute frequently more than 10% of the total hematopoietic population in individual animals, suggesting that the entire hematopoietic system may be repopulated by the progeny of fewer than ten cells.

Such small numbers would obviously make direct identification of these stem cells at various sites in the body impossible, but the demonstration by Trentin et al [3] that partially differentiated hematopoietic spleen colonies may contain these multipotential elements suggests that they may be present in any population of hematopoietic colony-forming units (CFU) derived from the bone marrow or other hematopoietic organs. These findings further indicate that the multipotential stem cell can circulate from one site to another, perhaps as small or large mononuclear cells indistinguishable from lymphocytes,[6] but they also raise fundamental questions about the earliest stages of hemic stem cell differentiation.

Relationships among Hemic Stem Cells

Many of the experimental data suggest that the pluripotent hemic stem cell can, under proper conditions, produce progeny that differentiate toward any of the five mature hemic elements (lymphocyte, monocyte, myeloid, erythroid, megakaryocytic), with the first stage of differentiation in each instance being a "limited" stem cell, committed to a specific pathway (Text-fig 1). This concept seems particularly well supported in the erythroid track, where the cell responsive to erythropoietin appears not to be the pluripotent stem cell, but rather a limited stem cell committed to erythropoiesis.[7] However, the precursor of the monocyte is not well defined as yet,[8] and there is also the possibility of an intermediate "marrow stem cell" between the pluripotent stem cell and the limited stem cells of the individual marrow elements. The existence of this cell in man is suggested by the observation in chronic granulocytic leukemia that the Philadelphia chromosome is present in myeloid, erythroid and megakaryocytic cells, but not in lymphocytes.

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                        ┌────────────────────────┐
                        │      Pluripotential     │
                        │  Lymphohematopoietic    │
                        │       Stem Cell         │
                        └────────────────────────┘
                          /    |      \    \     \
Limited
Stem Cells:    Lymphoid      Monocytoid    Erythroid  Myeloid  Megakaryocytic

          ┌─────────────────────┐
          │    Generator of     │  Monocyte      RBC   Granulocyte  Megakaryocyte
          │    Immunologic      │
          │     Diversity       │
          │                     │
          │ "Bursa"    Thymus   │  Macrophage
          └─────────────────────┘
             |          |  ◄──────── Antigen
    Immunoglobulin    Delayed
      Production    Hypersensitivity

    (B-Lymphocyte,  (T-Lymphocyte)
     Plasma Cell)
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TEXT-FIG 1—Possible interrelationships among hemic cells.

Clearly, the model indicated in Text-fig 1 will require modification as additional knowledge is developed.

The particular mechanisms that determine the direction of differentiation of the pluripotent hemic stem cell are discussed elsewhere, but one may consider here the question of whether the differentiation of the hemic cell once started along a particular track is capable of reversal to the stem cell stage and then diversion into a different track. Cells that are morphologically small lymphocytes, and presumably immunocompetent, can be transformed into "undifferentiated" blast forms when stimulated by antigens or by "nonspecific" mitogens such as PHA. However, it has not been possible to demonstrate definitely that these proliferating blast cells derived from functional lymphocytes can then be diverted into a myeloid-erythroid pathway of differentiation. Indications that the hematopoietic activity of bone marrow can be reduced by antilymphocyte serum [9] may suggest the possibility of such a conversion, and there have been attempts in cases of human aplastic anemia to initiate proliferation and differentiation of marrow elements by administering PHA *in vivo*.[10] The results of these attempts, however, have been equivocal, and limited efforts, including our own unpublished experiments, to increase the numbers of CFUs among a lymphocyte population from lymph nodes, blood, or thymus by stimulation *in vitro* with PHA have generally been unsuccessful.[4] It is apparent that further information is needed on what circumstances, if any, can cause cells that

have partially differentiated along one hemic track to revert to an un-
committed state and then differentiate in another direction.

Factors Affecting Lymphoid Differentiation

Closely associated with this problem of potential intraconversion
among partially differentiated hemic cells is the question of what factors
normally influence the differentiation of the multipotential hemic stem
cell *in vivo* in the direction of the lymphoid track and further differentia-
tion to immunocompetent lymphocytes. There has been considerable
experimental and clinical evidence in recent years indicating that in
prenatal life, and probably in postnatal life as well, the thymus plays an
extremely important role in the development of immunocompetence.[11]
It has been postulated that this may involve the "imprinting," in some
fashion, of undifferentiated lymphocytes from the bone marrow with the
capacity to function in immunologic reactions, particularly of the delayed
hypersensitivity type.

Another possibility is that the restricted immunologic specificity of
the lymphocyte is acquired before it reaches the thymus; this concept is
suggested in Text-fig 1 by the term *generator of immunologic diversity*
without specification as to site or mechanism. In this latter concept, the
thymus would simply modulate the already "committed" lymphocyte,
perhaps lowering its threshold to antigenic stimulation to make it more
readily responsive. The question of just how early in differentiation
lymphoid cells acquire lymphocyte-specific characteristics might be
approached by investigating the effect on hematopoietic spleen colonies
of antisera directed against specific surface antigens of immunocom-
petent lymphocytes (*eg*, theta antigen). The spleen colonies, as demon-
strated by Trentin *et al*[3] and Wu *et al*,[2] do not contain recognizable
lymphocytes or antibody-producing cells, but do include cells capable
of repopulating the immune system. If this latter capacity were elimi-
nated by the specific antisera, it would indicate that at least some degree
of immunologic differentiation occurs before developing lymphocytes
ever reach the thymus.

Whatever the exact nature of its action, the effect of the thymus on im-
munocompetent lymphocytes appears to be mediated probably through
a "hormone" produced by the epithelial cells of the gland, and there is
some evidence that this agent can act at distant sites in the body as well
as locally in the thymus itself.[11] A somewhat similar activity with respect
to the development of lymphocytes functional in immune responses in-
volving antibody production is mediated, in birds, by the bursa of

Fabricus, and perhaps through other "gut-associated" lymphoid tissues in mammals.[11]

Once this degree of differentiation has been achieved within the immune system through the acquisition of immunologic diversity and the action of the thymus or "bursa," the final differentiation of lymphoid elements to the stage of actual involvement in effector mechanisms of the immune response is mediated through antigenic stimulation (Text-fig 1). Each antigen may interact with a specific and distinct subpopulation of the total immunocompetent lymphocyte pool, and the evolution of these distinct subpopulations or immunologic "clones" within the animal has been the subject of intensive investigation and discussion in recent years.[12] Some of the experimental technics recently employed for the study of hemic stem cells have provided valuable opportunities to observe, in the adult animal, some aspects of the development of this immunologic diversity in the lymphocyte population.

Immunologic Differentiation of Lymphocytes in the Adult Animal

Several of the studies already described, which have provided evidence for a multipotential lymphohematopoietic stem cell, have also demonstrated the apparent capacity of the adult laboratory rodent to recapitulate completely, during recovery from sublethal irradiation, the generation of immunologic diversity that normally takes place during prenatal life.

The mechanisms by which an animal, during ontogeny, develops the capacity to respond specifically to a very large number of different antigenic stimuli has been widely debated since the "clonal selection" theory was first promulgated by Burnet.[13] Various hypotheses have been extensively treated elsewhere.[12,14] These concepts might be grouped into three general categories: (1) the view that immunologic diversity is completely genetically determined in the germ line; (2) the view that most of the diversity develops during ontogeny through some mechanism involving somatic recombination, mutation and selection and (3) the view that diversity and specificity is not finally determined until antigen interacts with the immunocomptent lymphocyte.

The hemic stem cell experiments in adult rats and mice under consideration here do not resolve these diverse points of view, but they do suggest that the same few primitive stem cells which can serve to repopulate the entire hematopoietic system of the irradiated rodent can also be the ultimate precursors of a completely restored and functional immune system in all of its normal complexity.

Trentin et al[3] showed that mice in which the entire hematopoietic

and lymphoid system had been repopulated from as few as four to 13 clonal lines of transplanted hemic cells were able to respond with high titers of circulating antibody to three different antigens: sheep red blood cells, *Salmonella* and bovine serum albumin. The data strongly suggested that differentiated lymphoid progency of a single cell were specifically responsive to the three different antigens.

Our own investigations with rats have produced similar indications. In the rat studies described earlier,[4] the clonal progeny of a single multipotential hemic stem cell, as indicated by chromosome markers, were able to respond immunologically to two different "alleles" of the major histocompatibility locus of the rat (AgB). There is now considerable evidence to indicate that the cells responding to various antigenic determinants in this system represent immunologically specific clones of unusually large size.[15] Thus, the conclusions of Trentin *et al*[3] with respect to antibody-producing mouse cells also appear to apply to immunocompetent rat lymphocytes responding to histocompatibility antigens.

The rat studies have recently been extended, in a preliminary fashion, to indicate that the same radiation-marked lymphocyte clone can react not only to different AgB specifications but also to protein antigens (*eg*, DNP-hemocyanin) capable of evoking delayed hypersensitivity reactions. Young male DA rats received sublethal whole-body irradiation, as in previous studies,[4] to produce clones of radiation-marked cells. The rats were screened for the presence of clones through the examination of at least 50 metaphase figures from lymphocyte cultures stimulated with PHA. A clone was considered to be present if the same readily recognizable abnormal chromosome markers (two or more) were present in at least 10% of the metaphases examined. Several rats with demonstrable clones were then immunized with 100 μg of DNP-hemocyanin (KLH) in Freund's complete adjuvant injected into two foot pads. Previous studies had shown that this antigen was capable of producing a delayed-hypersensitivity type of response in rats, and that peripheral blood lymphocytes from animals so sensitized would proliferate when challenged with antigen in culture. Five weeks after sensitization, peripheral blood lymphocyte cultures of four types were established from the irradiated sensitized DA rats: cultures containing DNP-KLH (10 μg/ml); mixed cultures containing either L/DA or BN/DA F_1 cells as antigen and cultures containing PHA.[4]

Table 1 illustrates the results obtained with 1 animal. The first PHA culture was that used in screening for clones; the second PHA culture was established simultaneously with those containing antigens. The

Table 1—Radiation-Marked Clones in Rat Lymphocyte Cultures Stimulated with Specific Antigen, Homologous (F₁) Cells or Phytohemagglutinin

Chromosome findings	Antigen (DNP-KLH)	Homologous Cells		Phytohemagglutinin	
		(Lewis/DA)	(BN/DA)	(First culture)	(Second culture)
Clone					
A	3*	11	11	13	12
B	9	1	5	0	2
C	4	4	2	2	2
D	2	3	4	4	5
E	2	2	2	0	1
Cells with other chromosomal abnormalities	12	6	7	12	7
Cells with no chromosomal abnormalities	18	23	19	19	21
Total No. of cells examined	50	50	50	50	50

* Number of cells observed in KLH-stimulated cultures with the three radiation-induced chromosome markers characterizing clone A (see text for details).

data demonstrate that in all cultures more than half of the metaphases examined had radiation-induced chromosome abnormalities, and that among these, four or five distinct clones, as previously defined, could be identified. Of particular interest are the clones labeled A and B. Clone A, which was characterized by three abnormal chromosomes including a minute metacentric (Fig 1), was present in approximately equal frequency (20–25% of the metaphases examined) in the two PHA cultures, established several months apart, and in the two mixed cultures, established against different histocompatibility alleles; but this clone constituted only 6% of the metaphases observed in the cultures stimulated with DNP-KLH. Conversely, clone B, characterized by two abnormal submetacentric chromosomes differing somewhat in arm ratio, was relatively rare in the PHA and mixed cultures (0–10%) but was the most frequent clone observed in the DNP-KLH cultures (18%).

Such fragmentary data do not permit definitive conclusions, but one might speculate that the similar frequency of different clones in the PHA and mixed cultures reflects the fact that the cells proliferating in these cultures represent a very large fraction of the total lymphocyte pool. The lymphocytes responding to PHA may include all of the immunocompetent thymus-derived lymphocytes, and those responding to

each of the AgB alleles comprise serveral percent of this thymus-derived population.[15] The cells responding to DNP-KLH in culture, on the other hand, may be derived, according to our present thinking, from a very small fraction (10^{-5} to 10^{-6}) of the thymus-derived pool. The very few cells initially specifically responsive to this antigen were expanded by *in vivo* immunization to a sufficient magnitude of population to produce a demonstrable proliferative response *in vitro;* but this population, having been derived from so few cells, might be expected to show a different distribution of radiation-marked clones than the total population represented in the PHA cultures.

More importantly, perhaps, these latest findings extend information on the postnatal regeneration of the immune system to another class of antigens that invoke a delayed hypersensitivity response and that have no known cross reactivity with the histocompatibility system.

Along with the earlier studies described, [3,4] the present data indicate that after sublethal irradiation, adult rats and mice can reconstitute their entire hematopoietic system, including the immune system in all of its normal diversity, from a handful of multipotential hemic stem cells. One must postulate that during the 15–30 days required for apparently complete recovery of these sublethally irradiated animals, the entire generation of immunologic diversity that normally takes place during embryogenesis is recapitulated. As the time span is similar for both gestation and recovery from irradiation, this conclusion does not rule out any one of the various hypotheses currently being advanced to explain the acquisition of diversity in the immune system, but certainly these findings in adult animals must be considered in approaching not only this problem but also the related question of the development of tolerance both to "self" and to foreign antigens under various circumstances.

Summary

A number of experimental studies indicate that in the adult rat and mouse there are pluripotential stem cells whose progeny are capable of differentiating not only into myeloid, erythroid, and megakaryocytic elements, but into immunocompetent lymphocytes as well. The entire hematopoietic system, including the immune system in all of its normal diversity, can be reconstituted from a handful of these stem cells during the few weeks required for a rat or mouse to recover from sublethal irradiation. Subsequently, distinct subpopulations of immunocompetent lymphocytes, responding specifically to different antigens, can be identified as having descended from the same hemic stem cell.

Factors controlling differentiation along various hemic tracks are still

poorly understood. Within the immune system, the important role of the thymus is gradually being clarified, and with the application of newer technics, such as the use of antisera directed against specific lymphocyte surface antigens, better understanding of the stages of immunologic differentiation may soon be forthcoming.

References

1. Barnes DWH, Ford CE, Gray SM, Loutit JF: Spontaneous and induced changes in cell populations in heavily irradiated mice. Progr Nucl Energy [Biol] 2:1–10, 1959

2. Wu AM, Till JE, Siminovitch L, McCulloch EA: Cytological evidence for a relationship between normal hematopoietic colony-forming cells and cells of the lymphoid system. J Exp Med 127:455–464, 1968

3. Trentin J, Wolf N, Cheng V, Fahlberg W, Weiss D, Ronhag R: Antibody production by mice repopulated with limited numbers of clones of lymphoid cell precursors. J Immunol 98:1326–1337, 1967

4. Nowell PC, Hirsch BE, Fox DH, Wilson DB: Evidence for the existence of multipotential lympho-hematopoietic stem cells in the adult rat. J Cell Physiol 75:151–158, 1970

5. Wilson DB, Silvers WK, Nowell PC: Quantitative studies on the mixed lymphocyte interaction in rats. II. Relationship of the proliferative response to the immunologic status of the donors. J Exp Med 126:655–665, 1967

6. Loutit JF: Versatile haemopoietic stem cells. Br J Haematol 15:333–336, 1968

7. Erslev A: Feedback circuits in the control of stem cell differentiation. Am J Pathol 65:629–640, 1971

8. McCulloch EA, Till JE: Regulatory mechanisms acting on hemopoietic stem cells: some clinical implications. Am J Pathol 65:601–620, 1971

9. Field EO, Gibbs JE: Cross-reaction of anti-lymphocyte serum with haemopoietic stem cells. Nature 217:561–562, 1968

10. Humble JG: The treatment of aplastic anemia with phytohemagglutinin. Lancet 1:1345–1349, 1964

11. Edited by V Defendi, D Metcalf: The Thymus: Wistar Institute Symposium Monograph No 2. Philadelphia, Wistar Institute Press, 1964

12. Mäkelä O, Cross AM: The diversity and specialization of immunocytes. Progr Allergy 14:145–207, 1970

13. Burnet FM: The clonal selection theory of acquired immunity. London, Cambridge University Press, 1959

14. Immune Surveillance. Proceedings of International Conference at Brook Lodge. Edited by RT Smith, M Landy. New York, Academic Press, Inc, 1970

15. Wilson DB, Blyth JL, and Nowell PC: Quantitative studies on the mixed lymphocyte interaction in rats. III. Kinetics of the response. J Exp Med 128:1157–1181, 1968
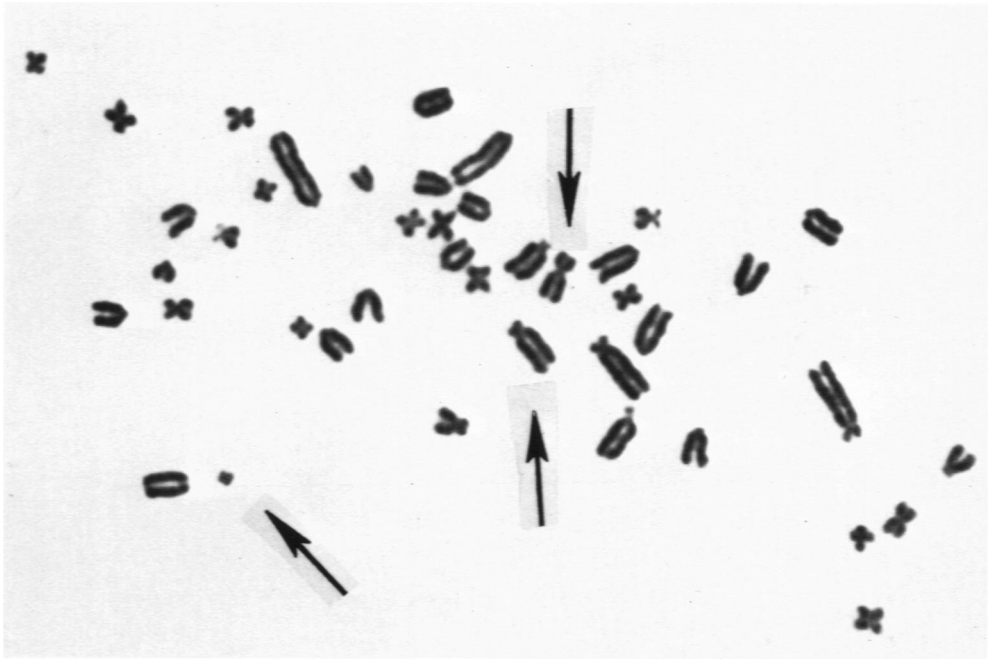
Fig 1—Metaphase from mixed leukocyte culture demonstrating the three abnormal marker chromosomes (*arrows*) that characterized cells designated as clone A in Table 1, considered to be descended from a common radiation-damaged stem cell precursor. Cells of this clone were induced to proliferate in culture by three different antigens (DNP-hemocyanin, Lewis/DA F$_1$ cells and BN/DA F$_1$ cells).

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