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Enhanced Histopathology of the Bone Marrow

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Abstract

Changes in bone marrow cellularity can be an indicator of systemic toxicity and, therefore, bone marrow should be included in the battery of tissues examined for enhanced histopathology. However, the majority of changes in the bone marrow that are observed in toxicology studies are a response to hematological changes or lesions elsewhere in the body. For this reason, a consideration of all tissue changes in the body is required in order to differentiate toxic effects versus physiological responses in the bone marrow. While enhanced histopathology involves evaluation of the separate compartments in each lymphoid organ using descriptive rather than interpretive terminology, bone marrow is unique in that it lacks specific compartments. Furthermore, identification of erythroid, myeloid, megakaryocytic, and stromal cells, plus adipose tissue and hemosiderin-laden macrophages, can be accomplished from conventional H&E-stained sections, but conclusive identification of lymphoid lineage cells is not likely. This limits the extent of initial enhanced histopathology on bone marrow and argues for the use of cytological preparations for more comprehensive assessment of potential immunomodulatory effects.

Keywords

Bone marrow; erythroid; myeloid; megakaryocytes; myeloid: erythroid ratio; maturation index

Introduction

The bone marrow is the largest primary lymphoid organ and is one location of antigen-independent lymphocyte development. It is also a secondary lymphoid organ because terminal antigen-induced lymphoid cell differentiation occurs within its microenvironment (Tavassoli and Yoffey, 1983). The regenerative capacity of most peripheral lymphoid organs depends on the pluripotent progenitor cells in the bone marrow. Changes in bone marrow cellularity can be an indicator of systemic toxicity and, therefore, bone marrow should be included in the battery of tissues examined for enhanced histopathology of the immune system. In fact, many compounds that target the bone marrow have been associated with profound alterations in immune function (Irons, 1985). Comparison of the cellular changes observed in the bone marrow should always be compared with the complete blood count. In any toxicologic study, the bone marrow from the treated animals should be compared with age- and sex-matched control animals due to the variation in normal cytological features that can be seen between sexes, strains and species of animals.

The majority of bone marrow changes that are observed in toxicological studies are the physiological responses of the bone marrow to hematological changes or lesions elsewhere in the body. By implication and because hematopoietic and lymphopoietic lineages share a

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common progenitor stem cell, it is reasonable to anticipate that systemic toxicity could affect multiple cell lineages. For this reason, a consideration of the health of the animal as well as all tissue changes in the body is required in order to differentiate primary (direct toxic effect) versus secondary (physiological response) effects on the bone marrow. The article by Travlos (2006) may be referred to for more detailed information on the normal structure, function and histology of the bone marrow.

Tissue Sampling and Preparation

Bone marrow is typically collected from the sternum, vertebrae or femur and can be processed for cellularity assessment by preparing conventionally fixed, paraffin-embedded sections of decalcified bone or isolated marrow casts or by preparing bone marrow smears. Since unequivocal identification of lymphoid cells in H&E-stained sections is problematic, one approach is to prepare bone marrow smears at the time of necropsy and, if there are cellular changes detected during examination of the H&E-stained tissue, quantitative and qualitative evaluation of Romanowsky stained bone marrow smears could be performed. Also, bone marrow cytomorphology can be difficult to assess on 5-micron tissue sections and, therefore, 3-micron sections might be preferable. Romanowsky stains might be more useful than H&E on these thinner sections. Bone marrow smears give the highest quality of cytological details with respect to cell morphology and maturation sequence.

Initial Evaluation

The evaluation of H&E-stained tissue sections can be used as an initial screening test for enhanced histopathology of the bone marrow. This evaluation would include an estimate of cellular density and a myeloid/erythroid (M:E) ratio. An increase or decrease in the numbers of megakaryocytes, adipocytes, stromal cells and amount of hemosiderin should be noted. Moreover, an indication of the presence and severity of necrosis, hemorrhage, fibrosis, granulomas, neoplasia, etc. should also be noted. Although the best indicator of immunomodulation would be a change in the lymphocyte population, lymphoid lineage cells are difficult to distinguish from many of the other nucleated cells in the H&E-stained bone marrow. If the M:E ratio appears to be altered, then a differential count on a bone marrow smear could be used to determine which cell line was altered and to give a quantitative assessment of that alteration. Subsequently, flow cytometry could be done to provide quantitative and immunophenotyping information about hematopoietic and lymphopoietic cell populations, although this would probably require subsequent studies to permit appropriate collection of samples.

An example of a checklist that can aid the pathologist during evaluation of the bone marrow is given in Table 1. This table is intended to be an example of a guideline that the pathologist can use during histological evaluation rather than a format for reporting lesions. The diagnoses listed in this table are descriptive rather than interpretive, consistent with the STP position paper: Best Practice Guideline for the Routine Pathology Evaluation of the Immune System (Haley et al., 2005).

Myeloid: Erythroid Ratio

The M:E ratio is a comparison of relative proportions of granulocytic and erythrocytic cells. In a cytologic preparation, it does not include lymphocytes. Since lymphoid cells are not readily distinguished from other nucleated cells in H&E-stained bone marrow, estimation of an M:E ratio from H&E-stained histologic sections would probably include both myeloid and lymphoid cells in the myeloid component. The M:E ratio generally has a mild myeloid predominance in most species. A change in the M:E ratio may be due to either the myeloid or erythroid cell lines. Comparison of the M:E ratio derived from the H&E-stained section with the CBC may

provide additional useful information for distinguishing which cell type is increased or decreased. It has been reported that the circulating lymphocyte count is not influenced by, nor does it reflect, changes in the lymphoid cell lineage in the bone marrow (Yoffey and Courtice, 1970). Obvious exceptions would include severe bone marrow cellular loss as occurs following exposure to cytostatic agents and increases in bone marrow and peripheral blood lymphocytes in lymphomas.

Maturation Index

The maturation index is the ratio between the number of proliferative phase cells to the number of maturation phase cells in the bone marrow and in mammals this number is typically 1:4 (Valli et al., 2002). Determination of this index is not considered a component of enhanced histopathology and would only be performed to more clearly define the nature of a hematopoietic defect. Careful examination of marrow histology and cytology must be done first in order to determine if there is an altered pattern of cellular maturation. Differential counts of cells in marrow smears could then be performed in order to better define the nature of the hematopoietic abnormality. For the myeloid series, the proliferative phase cells are the myeloblasts, promyelocytes, myelocytes and metamyelocytes, whereas the mature phase cells are the band and segmented neutrophils. For the erythroid series, the proliferative phase cells are the erythroblasts, prorubricytes, and rubricytes, whereas the mature phase cells are the metarubricytes. See Valli et al. (2002) for tables of normal differential counts and maturation indices for the Sprague–Dawley rat and for a summary of adverse marrow changes, including asynchronous maturation and dysplasia.

References

- Haley P, Perry R, Ennulat D, Frame S, Johnson C, Lapointe JM, Nyska A, Snyder P, Walker D, Walter G. STP position paper: best practice guideline for the routine pathology evaluation of the immune system. *Toxicol Pathol* 2005;33:404–7. [PubMed: 15805080]
- Irons, RD., editor. *Target Organ Toxicol. Ser.* Raven Press; New York: 1985. *Toxicology of the blood and bone marrow.*
- Jones, TC.; Ward, JM.; Mohr, U.; Hunt, RD., editors. *Hemopoietic System.* Springer-Verlag; Berlin Heidelberg: 1990. p. 1-27.
- Tavassoli, M.; Yoffey, JM. *Bone Marrow Structure and Function.* Alan R. Liss; New York: 1983.
- Travlos G. Normal structure, function and histology of bone marrow. *Toxicol Pathol* 2006;34:548–65. [PubMed: 17067943]
- Valli, VE.; McGrath, JP.; Chu, I. Hematopoietic system. In *Handbook of Toxicologic Pathology.* Haschek, WM.; Rousseaux, CG.; Wallig, MA., editors. Academic Press; San Diego, CA: 2002. p. 666-72.
- Yoffey, JM.; Courtice, FC. *Lymphatics, Lymph and the Lymphomyeloid Complex.* Academic Press; New York: 1970.

Figure 1.

Figures 1A–C are from a male F344 control rat. Figures 1D and 1E are from a male F344 rat treated for 90 days with 2,3-dibromo-1-propanol. These images illustrate multifocal areas of sternum bone marrow cellular depletion of the myeloid, erythroid and megakaryocyte cell lines (arrows). The remaining cells in these hypocellular foci are bone marrow stromal cells.

Figure 2.

There is a focal region of decreased cellularity in the sternal bone marrow (Figure 2A). Compared to controls (not shown), there were also increased numbers of erythroid precursors that appear as islands of small dark hyperchromatic cells throughout the sternal marrow (arrows, Figure 2B). Both of these lesions were present in 2-year-old female F344 rats exposed to a high dose of D&C red dye #9.

Figure 3.

Figures 3A–C are images of femoral bone marrow from 2-year-old female F344 rats exposed to trichlorfon. The arrows in Figure 3A show the multifocal nature of this lesion. The higher magnifications in Figures 3B and 3C illustrate the focal loss of cells, revealing the underlying stromal cells.

Figure 4.

These images are from a 2-year-old male B6C3F1 mouse exposed to 1,3-butadiene. There is diffuse and severe loss of hematopoietic cells in the sternum (Figures 4A and 4B) and cranium (Figure 4C). All cell lines had the same severe decrease in cellularity. The loss of hematopoietic tissue was so severe that this particular 2-year chronic study was terminated early.

Figure 5.

Figures 5C and 5D are images from a 2-year-old female F344 rat treated with 4500 ppm 2-methoxyethanol. There is a diffuse and mild decrease in hematopoietic cells with a corresponding increase in adipocytes in the femoral bone marrow. Figures 5A and 5B are from an age- and sex-matched control rat. The regional decrease in hematopoietic cells and a corresponding increase in adipocytes can be an age-related finding that is considered within normal limits. This example underscores the necessity of comparing suspected lesions in a group of treated animals with control tissues from age- and sex-matched concurrent controls.

Figure 6.

Figures 6A and 6B are from a control male B6C3F1 mouse in a 28-day subchronic study of 2',3'-dideoxycytidine-3'-azido-3'-deoxythymidine. Figures 6C–6E are from a treated mouse and illustrate dilated vascular sinuses, a marked treatment-related decrease in the dark, hyperchromatic erythroid precursor cells, and an increase in myeloid precursors.

Figure 7.

Figures 7A and 7B are images of femoral bone marrow from a male 2-year-old B6C3F1 control mouse. There is a normal ratio of myeloid to erythroid precursors (approximately 4:1) with normal maturation of both cell lines. Figures 7C–7E are from a mouse treated with phenolphthalein. These images show an overall decrease in the number of myeloid cells. Although some undifferentiated myeloid mononuclear cells are present, there is a marked decrease in the numbers of more mature segmented granulocytes. The megakaryocytic and erythroid cell lines appear to be unchanged. If desired, the maturation index of the myeloid cell line could be performed in this case to more clearly define the nature of the toxic alteration of cellular proliferation and maturation.

Figure 8.

Figures 8A and 8B are examples of femoral bone marrow from a 2-year-old male F344 control rat. Figures 8C–8G are from rats treated with methylene blue trihydrate. In this study, the bone marrow tissue in Figures 8C and 8D was diagnosed with 2+ increased cellularity whereas the bone marrow tissue in Figures 8E and 8F was diagnosed as 3+ cellularity. Figure 8G illustrates that this increased cellularity was diffuse and involved both the myeloid and erythroid cell lines. An increase in these 2 cell lines was associated with a corresponding decrease in adipocytes.

Figure 9.

Figures 9A, 9C, 9E and 9G are increasing magnifications of images of femoral bone marrow from a control 90-day-old female F344 rat with normal histological features typical of this group. Figures 9B, 9D, 9F and 9H are from a representative rat treated with 2-butanoneoxime. Note the diffuse increased cellularity of hematopoietic cells and the decreased numbers of adipocytes. The proliferative cell population is small with dark, hyperchromatic nuclei, indicative of the erythroid series.

Figure 10.

Figures 10A–10F are from a 2-year-old female F344 rat that was treated with erythromycin stearate. This is a broad-spectrum macrolide antibiotic that caused dose-related granulomatous inflammation in the liver, spleen and bone marrow. Figures 10A–10D are increasing magnifications of a focal region in the femoral bone marrow that has a decrease in the number of hematopoietic cells. Within this region are multifocal aggregates of histiocytes (arrows, Figures 10B and 10C) with occasional intracytoplasmic aggregates of pale yellow pigment (arrow, Figure 10D). Figures 10E and 10F are from a similar region in the same animal.

Figure 11.

These images are from a 2-year-old female F344 rat that was treated with 3000 ppm of 2-methoxyethanol. Figures 11A–11C are increasing magnifications of a focal lesion in the femoral bone marrow that was diagnosed as increased numbers of stromal cells. The macrophage is the predominant cell type in this lesion.

Figure 12.

Figures 12A–C are from 12-week-old female F344 rats treated with 2-butoxyethanol. Figures 12A and 12B illustrate diffuse severe femoral bone marrow necrosis in this rat after treatment with this compound that caused systemic intravascular thrombi. Figure 12C shows necrotic osteocytes as evidence of femoral bone infarction. The likely cause of the bone marrow necrosis is an intravascular thrombus within the central vein that is not present in this plane of section. Severe anemia was the major CBC abnormality in this animal. Figure 12D is from an unaffected animal in this study with normal bone marrow.

Figure 13.

Images 13A–13E are increasing magnifications of bone marrow from a vehicle-treated young adult (2–3 years old) cynomolgus monkey. The bone marrow in these images is considered within normal limits. Photomicrographs courtesy of Drs. Hans Harleman and Kathryn Bowenkamp.

Figure 14.

Images 14A–C are increasing magnifications of bone marrow from a young adult (2.5–4.5 years old) test-article treated female cynomolgus monkey. The bone marrow was evaluated after 7 oral doses of the test article. The bone marrow in these images was diagnosed as hypocellularity. For enhanced histopathology, you would indicate the decreased cellularity of all marrow cell lines with a study-specific severity grade. Photomicrographs courtesy of Drs. Hans Harleman and Kathryn Bowenkamp.

Figure 15.

Figures 15A–15E are increasing magnifications of bone marrow from a young adult (between 2 and 3 years old) male cynomolgus monkey that was treated with a test article. The bone marrow was evaluated after 12 days of IV dosing and showed slight increased granulopoiesis. In general, evaluation of bone marrow from all animals in a study, including controls and animals treated at all doses, allows for the determination of an appropriate descriptive term and severity grade for any particular bone marrow sample. For enhanced histopathology, the descriptive terminology would be “increased cellularity of granulocytes” with an appropriate study-specific severity grade. Photomicrographs courtesy of Drs. Hans Harleman and Kathryn Bowenkamp.

Figure 16.

The images in Figures 16A–E are increasing magnifications of bone marrow from a vehicle-treated 7–9-month-old male Beagle after 14 days of oral dosing. The bone marrow in these images is considered within normal limits. Photomicrographs courtesy of Drs. Hans Harleman and Kathryn Bowenkamp.

Figure 17.

These images are increasing magnifications of bone marrow from an 8–9 month-old male Beagle after 5 days of oral dosing with a test article. There is diffuse and severe decreased cellularity of the bone marrow. For enhanced histopathology, the particular cell lines that are depleted should be indicated as well as other processes such as necrosis, fibrosis, hemorrhage, etc. Photomicrographs courtesy of Drs. Hans Harleman and Kathryn Bowenkamp.

Figure 18.

Figures 18A–C are images of increasing magnification of bone marrow with decreased cellularity from a 7–9-month-old male Beagle after 14 days of oral dosing with a test article. The highest magnification (Figure 18C) shows that both the erythroid and myeloid cell lines are affected. However, there appear to be normal or increased numbers of megakaryocytes. Comparison with concurrent controls would help to determine if this cell line were increased or unaffected by treatment. Photomicrographs courtesy of Drs. Hans Harleman and Kathryn Bowenkamp.

Figure 19.

The images in Figure 19 are increasing magnifications of bone marrow from an approximately 7-month-old male Beagle that were obtained after 14 days of oral dosing with a test article. The diagnosis was severe increased granulopoiesis and decreased erythropoiesis. The low magnification views illustrate the diffuse hypercellularity of the bone marrow. The highest magnification (Figure 19E) shows the marked increase in granulocytes and decrease in erythroid cells. The number of megakaryocytes appears to be within normal limits. Romanowsky stained bone marrow smears and flow cytometry could be used to fully evaluate the lymphocyte population in this bone marrow. Photomicrographs courtesy of Drs. Hans Harleman and Kathryn Bowenkamp.

Table 1

Bone marrow checklist.

	Yes/Severity grade [*]	No
Increased (↑) or Decreased (↓) number of cells		
Myeloid		
Erythroid		
Megakaryocytes		
Adipocytes		
Reticular adventitial cells		
Macrophages		
Granulocytes (type)		
Hemosiderin		
Increased		
Decreased		
Necrosis		
Hemorrhage		
Fibrosis		
Granuloma		
Neoplasia		
Other		
Myeloid/Erythroid ratio =		

* One recommendation for a grading scheme would be 0 = normal, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.