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Project Title

Regulatory Mechanisms of Eosinopoiesis

Final Technical Report

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Final technical report

The major research effort during the past two years of the project has been directed to examine the response of the eosinophilic leukocytes system to changes in selected compartments of its cell population. The experimental approach has been to expose rats to total or partial body x-irradiation and determine the ensuing responses in the major hematopoietic sites and peripheral pools. The earlier data have already been reported and can be concisely stated as follows:

1) Whole body irradiation, 600 r. All major eosinophilic pools were affected with earlier recovery of the intestinal site followed by a slight overshoot of the marrow (118% of controls) by the 12th day after exposure. To be noted that the pattern of eosinophil recovery in the marrow was essentially identical with the decline and rise in the nucleated cell count.

2) Partial body irradiation, 600 r. Shielding of selected parts of the body (anterior half, posterior half, anterior 2/3, one hind limb) revealed some major differences in the response patterns of the eosinophilic and nucleated cells. Briefly, the nucleated cells of the shielded marrow showed essentially constant counts at the preirradation level: in contrast the eosinophilic cells reached values up to three times normal with a smooth rise beginning on the third day post-irradiation. In the exposed marrow, the nucleated cell count showed gradual recovery from the 3rd post-irradiation day onward at a much faster rate than the eosinophil counts of the same marrow. The overall pattern emerging from all the studies where various forms of shielding were tried, was thus consistent

with the conclusion that the shielded marrow was responding to a specific eosinophilic stimulus, possibly originating in the irradiated areas with a time course independent of the regenerative pattern of the other nucleated elements of the marrow. The more recent experiments have been directed to test some of the factors which could influence the marrow response and offer a clue to its mechanism. These were in brief, the approaches and the results. Head and limb shielded rats were exposed to different doses of radiation (150, 300, 450, and 600 r). The effects on the protected and irradiated marrow were generally opposite and in proportion to the dose: (hyper) eosinophilia of the shielded marrow increasing with the dose, while the repopulation rate of the exposed limbs decreased. These opposite trends were reflected in the eosinophil in shielded/eosinophils in irradiated ratios at day 12 which varied as follows (eosinophil counts referred to controls = 100):

after 150 r	= 157/133	ratio = 1.18
300 r	= 130/54	= 2.40
450 r	= 184/46	= 4.00
600 r	= 290/30	= 9.67

These data indicate a vicarious function of the protected marrow heightened by the severity of the radiation-induced damage, an inference also supported by the results of the previous series where the area being irradiated, rather than the dose, was increased from half to approximately 2/3 of the body surface.

Shielding of the abdomen in addition to the head and limb did not abolish but decreased the shielded marrow eosinophilia at days 11 and 14 to about one half. Evidence was also sought that the marrow eosinophilia is not simply due to a block of release or lack of distribution to peri-

pheral pools diminished in their volume by radiation injury. The latter possibility is unlikely because of the absence of blood eosinophilia and the difference in the timing of the effects: therefore, the question can be reduced to the evidence pro or con a release block. Two avenues were tested: the first consisted of determining the ratio of immature to mature eosinophilic cells in the shielded marrow. Data were obtained for the interval day 3 - day 11 during which the immature/mature ratio lay within the range seen in control animals, without apparent relation to the early decline or the late rise in eosinophil counts. The second approach was to administer dexamethasone (5 mg/kg), a procedure known to block eosinophil release doubling the count for these cells during the next 24 hours. Animals thus treated on day 10 post-radiation exhibited 24 hours later a shielded marrow eosinophil count one and one-half times higher than the irradiated non-injected rats. The conclusion can be drawn that a substantial number of eosinophils were still released from the marrow. The lack of detectable shift in the immature to mature ratio and the progressive concurrent gain in peripheral pools titers for the phospholipase are consistent with this view. Another group of experiments analyzed the late trends in marrow and blood: the results showed a progressive trend of recovery in the exposed marrow which actually reached (hyper) eosinophilia levels by the 22nd day. At this time the readings were the same in both protected and exposed marrow and declined in similar fashion over the ensuing week. Blood eosinophils also rose to twice the normal range but with a 2-3 day delay, and remained above normal at a time when marrow values had already returned to baseline levels.

The last group of experiments was undertaken with the purpose of relating these experimental findings with the clinical condition of increased eosinophilic blood counts reported as a relatively common occurrence after radiation treatment (Radiation Related Eosinophilia = RRE), which is seen by some authors as a symptom of some favorable prognostic significance in tumor therapy. From the clinical standpoint, it is worthy of note that RRE occurs almost exclusively in association with irradiation of the pelvis: consequently, the most immediate and traditional inference has been to envisage an eosinopoietic stimulus originating in the abdominal viscera by a variety of mechanisms ranging from local inflammatory reactions to immune or autoimmune phenomena. Others, however, have pointed to the fact that in most instances the bones of the pelvis, which are estimated to contain a large fraction of the total marrow (up to 40%), are included in the field of treatment and might contribute to the response. Repeated treatment would magnify such effects by creating a continuing demand on the non irradiated marrow. In fact, Muggia (Muggia et al., Oncology 27, 118, 1973) has stated "eosinophilia was more frequent in patients receiving radiation to large areas of bone marrow" without a correlation between RRE and granulocyte counts, thus suggesting independence of the responses of the two cell lines, as seen by us in the studies with partially shielded rats. In this light, experiments were performed to determine if irradiation of abdominal viscera is essential in terms of eliciting post-radiation marrow eosinophilia. Two sets of experiments were designed: the first involved irradiation of approximately 2/3 of the body with shielding of the abdomen and one limb. The effects in the exposed and shielded marrows did not differ substan-

tially from those seen when the abdominal area was not protected. The intestinal eosinophil pool was not affected thus confirming the effectiveness of the shielding. The second experimental setting involved irradiation of the whole body minus the abdominal area and sampling of the marrow of the pelvic bones to ascertain if the protected marrow exhibited a post-irradiation eosinophilic response. This experiment required the development of adequate techniques of bone marrow sampling from the iliac crest and a series of control tests to warrant the accuracy of the methodology employed. The results of this part of the study showed the expected pattern of decline and late recovery for both eosinophil and nucleated counts in the exposed marrow. The response of the shielded pelvic marrow is shown in Table 1 and consisted of a progressive rise to values more than twice the pre-irradiation level with an apparent peak at the 12th day in a pattern which closely resembled that previously seen in rats shielded over one of the hind limbs.

TABLE 1

Eosinophil Counts (a) in Shielded Bone Marrow of Rats Exposed to X - Irradiation (600 r)

	DAYS AFTER IRRADIATION						
	0	3	7	10	12	14	17
Eosinophils	60.400	52.000	53.300	97.100	153.500	76.200	54.000

@ = Eosinophils per mg of bone marrow

No major changes were present in the intestinal and splenic values confirming the adequacy of shielding. These findings lend strong support to the thesis that the stimulus to eosinopiesis is more directly linked to the irradiated marrow then to the irradiated viscera, at least in the experimental model considered here. The correctness of this view remains to be confirmed by additional evidence involving demonstration of the specific stimulus, possibly by transfer to syngenic recipients and eventual identification of its chemical and biochemical characteristics. The data summarized here will become part of a publication now being prepared and will be communicated in a radiation research meeting in the near future.