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FINAL PROGRESS REPORT

U.S.A.I.D Grant DAN-5053-G-SS-7024-00 titled, "FUNCTIONAL PROPERTIES OF IMMUNE CELLS AFTER ONTOGENIC EXPOSURE TO BENZO(a)PYRENE", Principal Investigator, Paul Urso, Ph.D., Morehouse School of Medicine.

A. SUMMARY OF ACCOMPLISHMENTS.

1. Quantitative Analysis of immunocompetent cells.

In benzo(a)pyrene (BP) exposed mice, after we originally demonstrated: a) quantitative changes in cells expressing Lyt antigens in fetal, postnatal (progeny up to 30 days, maternal up to 10 days), and maternal lymphoid tissue (fetal liver, thymus and spleen); b) susceptibility to growth of a syngeneic fibrosarcoma in the progeny (Urso and Johnson, 1987a, 1988a), we also observed the presence of cells expressing L3T4 (T4) antigens in the fetal liver (FL) which were 3- to 4-fold higher than those expressing Lyt 1 antigens and about equivalent to those demonstrating Lyt 2 antigen (Urso et al, 1988a,1989ab). These data suggest the existence of several types of cells in the FL (and fetal thymus) hitherto uncharacterized, including a T4+1- cell.

Analysis for various cell types by sequential cytotoxicity and indirect immunofluorescence (see detailed methodology below) revealed additional modifications in progeny lymphoid tissues. Most surprisingly, we observed double positives in FL (17-19 da gestation) including 1+2+ and T4+2+, the latter being 2.5-fold greater than the former for BP-FL, and when compared to CO-FL, these cell types were 1.5- to 2.5-fold lower. The same kind of pattern was seen for the fetal thymus. Other cell types detected included T4+1+, T4+1-, and 1-2-. When BP-fetal thymus was compared to CO-fetal thymus, the dramatic changes were a 2-fold decrease in double positives and a 1.5- to 3.5-fold increase in the other cell types, most striking being the increase of double negatives (3.5-fold). A manuscript is in preparation describing these data and their significance (Urso et al, 1989c).

Since maternal-fetal associations are influential in development of progeny immune status, additional quantitative studies were performed on maternal blood cells during pregnancy and after parturition following BP injection. Briefly, our results showed a preferential depletion of peripheral blood lymphocytes, a situation which could have significant consequences in establishing immune function in the progeny (Urso et al, 1988b). We are currently in the process of identifying, by immunofluorescence and cytotoxicity, the kinds of lymphocytes affected .

2. Functional characteristics of fetal liver cells.

We have previously shown that BP-FL cells suppressed proliferation of T cells reacting against histoincompatible spleen cells in a mixed lymphocyte culture (MLC), while CO-FL cells augmented proliferation, actions which appeared to be mediated by cells expressing Lyt 1 (Urso and Johnson, 1986,1987b,1989ab). Since the FL preparations consisting of Lyt 1+2- and Lyt 1-2+ also contained other cells of immune potential, it is possible that such cells may also participate in immune activity (e.g., macrophages). It is known that nylon wool and glass wool fibers can remove macrophages from lymphoid cell preparations. Effluent BP-FL or CO-FL cells abrogated suppression and augmentation, respectively, while the fiber adherent cells restored these functions; when effluent and adherent cells were combined, BP cells suppressed and CO cells augmented proliferation (Urso et al, 1988a, 1989ad). These data suggest that FL macrophages are involved in these functions, but since some T cells are also trapped in the fibers, the possibility of interactions between these two cell types exists. Currently, we are

phenotyping the adherent and effluent cells for more precise identifications. Three manuscripts are in preparation for publication (Urso and Johnson, 1989ab, Urso et al, 1989d).

For functional studies with different cell types, we are developing panning procedures for obtaining purified cell populations. In some preliminary isolations, We have obtained 85-90% T cells from adult spleen with polyclonal anti-θ. Thus, since this procedure appears feasible, for more purified T and macrophage cell populations (>95%; Wysocki and Sato, 1978, Sprent and Schaeffer, 1985), panning will be done with monoclonal antibodies (e.g., anti-Thy 1, anti-Lyt 1, anti-Lyt 2, anti-L3T4, and anti-mac).

3. Functional characteristics of the thymus.

In earlier studies we showed that the splenic allogeneic mixed lymphocyte response (a function which measures T helper cell capacity) is depressed in progeny exposed to BP in utero (Urso and Gengozian, 1984); is inhibited by BP in adult virgin spleen cells in vitro (Urso et al, 1986), and maternally suppressed during pregnancy and 1 wk postpartum. Maternal splenic syngeneic response is also suppressed (Urso and Johnson, 1988a). In view of this, maternal-fetal associations, and the central role of the thymus in supplying peripheral lymphoid tissue with mature T helper (L3T4) and effector (e.g., cytotoxic and/or suppressor) cells (Lyt 2), we measured the ability of progeny and maternal thymus cells to undergo syngeneic or allogeneic mixed lymphocyte responses (MLR) during gestation (pregnancy) and postnatal (postpartum). To extend the data submitted in our previous report, we have observed no differences in the maternal syngeneic MLR during pregnancy and early postpartum (1 wk), but the allogeneic response of maternal BP-thymus cells was reduced 3- to 100-fold. Although the BP-progeny syngeneic response was not affected during gestation, and only slightly suppressed at 1 wk post-birth, a dramatic suppression was observed from the 2nd to the 4th wk. On the other hand, the allogeneic response was enhanced during gestation, but suppressed after birth (up to 4 wk). A manuscript is in preparation for publication (Urso et al, 1989e).

4. Other functional analyses.

A. Natural killer cell (NK) and cytolytic T lymphocyte (CTL) activity. Data obtained from Dr. N. M. Ponzio (New Jersey School of Medicine) indicated a depression of NK activity in progeny up to 8 wk after birth, but no difference after this time. Some decrease in CTL was observed early after birth (up to 5 wk). Additional data need to be obtained for more conclusive information.

B. We are also attempting to explore the ability of lymphoid tissue of progeny to produce interleukin-2 (IL-2). Up to now we have standardized the proliferative response of CTLL-2 cells (a cytolytic T cell clone dependent on IL-2 for growth). Using ^{3}H-thymidine, it appears that optimal proliferation of this cell line occurs when 10^5 cells are obtained 4 days after culture in the presence of 20% IL-2 that was produced in normal rats. We are in the process of testing supernatants of thymus, spleen and fetal liver cells of experimental and control progeny cultured with concanavalin A for IL-2 in supporting growth of CTLL-2.

C. We are also initiating experiments to obtain macrophages from maternal peritoneal cavity to test their functional capacity (e.g., phagocytosis and killing of opsonized sheep red blood cells), including whether or not they have suppressor action against T helper cells proliferating against allogeneic responder cells in a standard MLR.

5. Use of these research findings by U. S. A. I. D.

These data illustrate the striking modifications that can occur in cells of the defense mechanism after exposure to the carcinogenic environmental pollutant BP. It is not unlikely that similar modifications can be induced by other environmental pollutants known to affect the immune system. The extreme complexity of the immune system, including the network of cellular and humoral components and interactions that must remain homeostatically intact, and the alterations that can occur in this system, particularly those initiated during its developmental stages, warrants precise in depth identifications of this network so that alterations can be pinpointed and subsequently dealt with. This will provide important groundwork for establishing therapeutic measures that can improve health conditions, not only for the poor of developing countries who are exposed to excessive amounts of this (BP) and other pollutants which drastically alter immune status, but also for the population world-wide.

B. DETAILED STATEMENT OF THE RESEARCH.

The problem addressed in this research is that poor health conditions, increasing in Third World Countries and attributed to exposure to environmental pollutants, can affect women, particularly those of child bearing age. As a result of this exposure, harmful situations also can develop in the progeny. The research question of foremost importance is how in utero exposure to the environmental pollutant, (BP) modifies the production and functional capacity of immunocompetent cells, not only those of the progeny but also those of the mother, and how this affects the differentiation potential of components in this system. Ontogenesis of immune components along established pathways is important for combating deleterious agents. Disruptions in these components can lead to severe deleterious health effects such as respiratory diseases (particularly in children) and tumorigenesis.

1. Detection of immunocompetent T cell types in lymphoid tissue.

The kinds of cells present in the FL, thymus, and spleen (progeny and maternal), and their frequency are important in eventually establishing immune potential in peripheral lymphoid organs. Detection of these cells after BP insult is important for 2 reasons: a) insight may be obtained on the cellular pathways needed to establish an efficient adaptive system; b) identifications of alterations that can occur and that must be modified also need to characterized to maintain normal progression of the system. We have used two methods for detection of cells expressing T cell antigens. These are described briefly as follows:

Sequential cytotoxicity is as follows: a 1st preparation of cell suspension is incubated with the 1st monoclonal antibody (mab) (e.g., anti-Lyt 1) on ice, followed by C' @ 37° C. A small amount of this is added to trypan blue (dead cells carrying Lyt 1 antigen stain blue), the cell preparation fractionated on ficoll-hypaque (to remove those dead), interface cells incubated with the 2nd mab (anti-Lyt 2) + C', and the frequency (%) of dead cells (trypan blue uptake) recorded. A second preparation equal in cell number to the first, is subjected to reciprocal treatment with mab's (anti-Lyt 2 followed by anti-Lyt 1). The second incubation will detect frequency of single positives; subtracting this and

frequency of null cells (average from the 1st and 2nd preparations) will yield the frequency of double positives.

Indirect immunofluorescence for quantitative detection is as follows: cells will be incubated with mab, followed by goat anti-mab conjugated with fluorescien isothiocyanate (FITC) specific for 1 of the markers (e.g., 1^+ mouse IgG2a) and goat anti-mab conjugated with tetramethylrhodamine isothiocyanate (TRITC) specific for another marker (e.g., 2^+ mouse IgG3) (the mab's do not cross-react). In our tests with normal thymus, we detect cells fluorescing green only mingled with red fluorescing cells and with cells fluorescing both green and red with the appropriate exciter and barrier filters in place. For double positive cells, interestingly, green and red fluorescence are in the form of discrete particles, i.e., those that fluoresce only green (i.e., the Lyt 1 marker) on the same membrane with those that fluoresce red only (i.e, the Lyt 2 marker) (unpublished observations). This is also true for the L3T4 marker. For T4+ cells, mab rat anti-mouse T4 (from clone Gk 1.5) is used, followed by goat anti-rat FITC or TRITC that is not cross-reactive with mouse Ig. The frequency of double positives is as described by others (Scollay and Shortman, 1985), i.e., 80-90% double positives and 5-15% single positives. When using FL and mouse mab against Lyt 1 or Lyt 2, IgM and IgG on B cells is blocked with goat anti-mouse Ig (reacting against μ and γ chains) before application of mab (which does not cross-react against goat Ig) and goat anti-mouse Ig FITC or TRITC.
To block for Fc receptors when using mouse mab, normal mouse Ig is used followed by goat anti-mouse before applying mab anti-Lyt and fluorescing goat anti-mouse. for the Lyt antigens mab rat anti-mouse Lyt eliminates blocking the Fc receptors.

In 10 experiments by sequential cytotoxicity for the BP-FL the Lyt 1/Lyt 2 ratio was <1 (0.88), confirming our original results (Urso and Johnson, 1987a), while in the CO-FL the ratio was 1.4. This was confirmed by indirect immunofluorescence in 3 experiments (ratio of 0.60 vs 2.00, respectively). Most surprisingly, by cytotoxicity we detected 5 of 9 BP-FL (each value was a pool of FL's from the siblings of 1 mother) containing a mean of 3% for the quantity of 1+2+ cells, comparable to the mean value obtained by indirect immunofluorescence (mean of 3% for 8 experiments, all of which gave double positive cells). For the CO-FL, 3/5 showed a mean of 4% for the 1+2+, while by fluorescence the number was 2.5-fold higher for pooled FL cells from siblings of 8 mothers. We did not attempt sequential cytotoxicity with rat anti-mouse L3T4, but by immunofluorescence the ratio of double positive L3T4+2+ (BP/CO) was 0.67 (8%/12%). Most dramatic was the detection of other cells expressing one or the other T cell antigen. Thus both BP- and CO-fetal livers showed similar amounts of T4+1- cells (17% vs 16%) and a slightly lower (albeit very low) number of T4-1+ in the BP-FL (1.3% vs 2.3%).

For the fetal thymus the results were very unexpected. The most dramatic was a 3.5-fold increase in 1-2- (double negative cells) in BP-fetal thymus (42% vs 12%, three experiments) by sequential cytotoxicity (presently we are quantitating the number of double negatives by immunofluorescence, including T4-2-). In addition, by immunofluorescence, we detected decreases in T4+2+ (44 vs 71%), T4+1+ (23 vs 42%), and an increase in T4+1- (67 vs 44%) and T4+2- (35 vs 21%). However, as in our earlier data (Urso and Johnson, 1987a), the ratio of 1+/2+ was >1.00 as was the ratio of T4+/2+. (Two to 9 experiments were performed for these analyses which are continuing.)

The significance of the cell profiles in the FL awaits functional experiments with these cell populations isolated by panning [and, perhaps more precise, by

the fluorescence activating cell sorter (FACS)] (see below on functional aspect of the FL). In the fetal thymus, however, the toxic effects of BP cannot be the only explanation for the changing patterns of cell types observed. It appears that disruption by BP involves changing pathways for differentiation of these cells, i.e., into more T4+1- and T4+2-, perhaps directed from the increase in double negatives (e.g., 1-2-, and presumably T4-2-). Experiments will be designed to test this concept.

2. Functional activity of the FL cells.

We have shown that BP-FL cells suppress T cell proliferation of a standard mixed lymphocyte response, while CO-Fl augment it; further, BP-FL expressing Lyt 1 appear to mediate suppression, while the CO cells mediate augmentation. This strongly suggests a functional reversal of these cells, perhaps mediated by cells expressing Lyt 1 antigen. However, since macrophages (adherent cells) are present and functional in the FL, it is essential that we characterize participartion of these cells. As a first step we fractionated FL cells on nylon wool or glass wool, the former removing macrophages and B cells and the latter mostly macrophages. The results we obtained with nylon wool and glass wool were very similar, and only the nylon wool data will be described in this report. Our methodology was to apply 75 to 100×10^6 FL cells onto the fibers and recover the effluent and adherent cells. The recovered cells were then fractionated on ficoll-hypaque and the interface cells cultured in concentrations of 0.25×10^6 with 1×10^6 syngeneic splenic responder cells mixed with 1×10^6 allogeneic stimulator cells. When effluent FL cells were mixed with adherent FL cells the concentration was 0.5×10^6 (1:1 effluent with adherent). The cultures were incubated at 37° C under 5% CO_2 in a humidified atmosphere, pulsed with ^3H-thymidine at 4 or five days, recovered 16-20 hr later, and ^3H incorporation measured in a liquid scintallator. Results were expressed in counts per minute (CPM) and % of the standard mixed lymphocyte response (MLR). Although the CPM of the standard varied (from 23 to 37×10^3), the change in CPM with BP- or CO-FL cells was consistent for these groups to allow expression of the data in terms of % MLR. In 6 experiments for interface cells from unfractionated (not placed on nylon wool) CO-FL the MLR was increased 2-fold (e.g., standard MLR CPM was 23-37×10^3, while CPM with FL was 2-fold higher). In 5 experiments for BP-FL the MLR was reduced by 35%. In 12 and 11 experiments, respecitvely, this pattern was completely reversed by interface cells obtained from the nylon wool effluent. Nylon wool adherent cells restored the original patterns (4 experiments) except that CO-FL adherent cells did not augment proliferation but restored it to standard levels for the MLR. When effluent and adherent cells were cultured together (4 experiments) the CO cells significantly augmented proliferation, but not to the similar levels seen for whole FL cells (i.e., the increase was 30%, not 100%), while the BP-FL mixtures not only continued to show suppression, but the suppression was more severe (60 to 70%). If Lyt cells of CO-FL are augmentors, the results suggests that either they a) augment in the presence (or absence) of macrophages, or b) augmentor cells are trapped on the fibers allowing for suppressor cells (not macrophages) to express themselves in the effluent (natural suppressors?). It does not seem likely that macrophages are augmentors, but it is possible that they secrete interleukin-1 which enables T cells to enforce the augmentor effect in the presence of allogeneic tissue antigen. The nature of the augmentor cells (and the suppressors in the effluent) is under

investigation (see below). With respect to the data obtained for the BP-FL cells, it seems likely that suppression can occur with macrophages, either alone or with other Lyt cells. (B cells are not retained on glass wool fibers). At any rate, the data supports and appears to confirm the functional reversal of FL induced by BP. A manuscript is in preparation and will be submitted to Cellular Immunology shortly (Urso et al, 1989d).

To more precisely identify the cells in these roles we are phenotyping effluent and adherent cells. It is known that splenic Lyt 1 cells are trapped on nylon wool fibers and appear to be cells which are a kind of helper that is involved in proliferative activity of B cells and are Ia^+ (Tada et al, 1978). It is of significance that the T helper cell population appears to be polymorphic (Swain, 1988). Thus, the FL needs to be examined for such Ia^+ Lyt 1 cells that may also cooperate with macrophages (and/or other T cells) to induce proliferation of responder T cells in the MLR and whose expression may be reversed (again in cooperation with macrophages) by BP. We intend to use panning (briefly explained in the SUMMARY) to isolate specific T cell types and macrophages and, hopefully, the FACS for confirmatory and more precise data. We will negotiate with scientists from Emory University or The Centers for Disease Control in Atlanta, to arrange for use of the FACS. Both institutions have active programs that utilize the FACS for isolation of T cells.

3. Thymic mixed lymphocyte response.

To integrate the results obtained with respect to the detection of immunocompetent cells in the FL and fetal thymus, and the functional characteristics of FL cells, we have studied the functional activity of thymic cells in terms of recognition of self and non-self. Since it has been amply documented that the thymus is the central organ of T cell differentiation, study of its functional characteristics during ontogenesis in the progeny and mother and the effect of BP-exposure may provide insight on the changes induced in splenic maternal syngeneic and allogeneic MLR, modification of thymic and liver cells expressing T antigens, and the functional disorientation in the FL. We employed thymic cell doses of 1.00, 0.50, and 0.25×10^6 as responder (R) cells cultured with equal amounts of syngeneic or allogeneic splenic stimulator (S) cells (R:S of 1:1). The stimulators were inactivated with mitomycin C and washed thoroughly to remove this toxic reagent. The cultures were incubated at 37°C, under 5% CO_2 and humidity, pulsed with 0.5 uCi ^{3}H-thymidine on 3, 4 or 5 da, cells recovered 16-20 hr later and radioactivity determined in a liquid scintillator.

For the progeny thymus, the results were as follows: In a limited number of experiments (1-3 representing individual progeny from different litters) there appeared to be no change in the syngeneic response during gestation, but after birth (3-9 experiments), proliferation of thymic cells was depressed particularly evident at 14 da (3- to 7-fold suppression at all R doses used). Although the CPM were low ($0.03-2.00 \times 10^3$) a stimulation index (SI) of >1.2 [e.g., SI=CPM R + S/CPM of R + TCM (tissue culture medium)] was considered as a response. The allogeneic response of thymic cells was enhanced during gestation (17 da and particularly at 19 da), reaching SI values of >10 (e.g., CPM of R + S = 20×10^3/CPM of R + TCM = 2×10^3). At 1 to 4 wk after birth, suppression occurred which was most dramatic from 2 to 4 wk.

In the BP-mother the functional activity during 17 da of pregnancy and 1 wk

postpartum was not different from the control. Additional studies are in progress for time intervals similar to those measured in the progeny. The allogeneic response during pregnancy (17 and 19 da) was decidedly suppressed, particularly at 19 da. Thus, for example, at 3, 4 and 5 da of culture, the SI was 20-, 35-, and 100-fold lower than that for the CO thymus cells, respectively (5 to 7 experiments) at a dose of $1x10^6$ R cells. A similar pattern was seen at 1 wk postpartum. CPM recorded ranged from $0.04x10^3$ to $1.0x10^3$ for R + TCM, and from $0.8x10^3$ to $100x^3$ for R + S.

It is clear from the data that BP induces functional disorientation of cells in the thymus by altering the ability of the cells to respond by proliferation against self and non-self (allogeneic) tissue antigens. This modiification cannot be attributed entirely to the toxic effects of BP since the proliferative response against allogeneic antigen was markedly enhanced during gestation and only slightly reduced at one wk after birth. It may be significant that the frequency of T4+1-, T4+2-, and 1-2- was enhanced, as shown by immunofluorescence and cytotoxicity (see above), a situation that may account for the enhanced MLR. Although differentiation of thymic stem cells is believed to be a pathway that involves double positive intermediate cells (>90% of which die in the thymus cortex) into mature (and functional) single positives, the evidence is largely circumstantial and indirect. Since we show depressed double positives in the BP-thymus, it is tempting to speculate that there is direct maturation of double negatives into mature functional cells. If this interpretation is correct, mature functional cells would probably not only be decreased, but also would be functionally defective in recognizing and responding against self (syngeneic) and non-self antigen in the postnatal thymus. It is clear from the data that both responses (self and non-self) are defective. The defect in recognizing self class II major histocompatability antigens may be responsible for unresponsiveness against non-self. Experiments to test these possibilities will be designed.

The profile in the maternal thymus is different from the progeny. Although functional (MLR) disorientation also occurs there is no effect on the syngeneic MLR at the 2 intervals test (17 da pregnancy and 1 wk postpartum). This is not only unlike the progeny, but also different from the splenic syngeneic MLR which was decidedly depressed during pregnancy (15-19 da) and 1 wk postpartum (Urso and Johnson, 1988a). On the other hand, the allogeneic thymic MLR at 17 and 19 da of pregnancy and 1 wk postpartum was markedly depressed. The functional dysfunction in the maternal thymus may explain the suppressed splenic maternal MLR by not supplying normal, functionally mature T lymphocytes to the peripheral lymphoid tissue, i.e, the T helper cells emigrating from the thymus are defective. In terms of maternal-fetal associations, the lack of parallelism mainly in the allogeneic response, appears to preclude a maternal influence on development of the immune system in the progeny. However, it is believed that maternal factors play a significant role in survival of the fetus, and a lack of these may lead to an enhanced capability of the fetus to protect itself, i.e, by an enhanced allogeneic MLR representing a compensatory mechanism. On the other hand it is also possible that a dysfunctional maternal thymus may not supply essential ingredients (e.g., thymic hormones) to maintain the functional integrity of the offspring after birth [syngeneic and allogenic postnatal progeny thymic MLR and splenic allogeneic MLR (Urso and Gengozian, 1984) are subnormal]. Tests for these possiblities require additional analyses of maternal circulating peripheral blood lymphocytes, placental cell populations, and maternal-fetal lymphocyte

migrations (to name a few).

4. Acitvity of natural killer (NK) and cytolytic T lymphocyte (CTL) cells.

In previous data obtained from Dr. N. M. Ponzio, New Jersey Medical School, some depression was obtained for these cells in the spleen of progeny and mother at 5 wk after birth (unpublished observations), while at 10 and 18 wk their activity did not appear to be different (in a limited number of experiments involving 1-3 anmimals). Recently we have obtained additional data for NK cells reacting against Yac-1 ($H-2^{a/a}$) lymphoma cells in a ^{51}chromium (^{51}Cr) release assay. BP or CO effector cells are cultured with ^{51}Cr-Yac-1 cells and incubated at 37°C for 4 to 8 hr and ^{51}Cr release detected in a gamma counter. The Effector cells are normal or treated with poly I:C (enhances NK activity). The data for release are expressed in lytic units (1 unit = 30% cytolysis). Depression of NK activity was obtained in 3 wk old BP-progeny, particularly evident when comparing BP and CO spleen cells after poly I:C stimulation (3 vs 4 units, respectively - 4 determinations), which was most dramatic at 8 wk (3 vs 8 units). However, at 19 and 25 wk no differences were oberved. For CTL reacting against ^{51}Cr labeled P815 cells a slight suppression (25%) of cytolysis was observed up to 5 wk, but none thereafter (only 2 determination per interval). Other than there being some early disruption of NK activity, additional data must be obtained before definitive conclusions are made. Experiments are in progress to provide this data.

C. ACCOMPLISHMENTS REFLECTING GOALS.

We have provided important data on the likely participation of macrophages in the suppressor effect of the FL from BP-exposed progeny (and augmentor effect from CO-progeny), that these cells likely interact with other cells of the FL expressing T lymphocyte antigens, and that cellular mediators are likely to be cells expressing Lyt 1 (and perhaps Ia^{k+}). We still need to determine which of the cells are suppressors or if a combination of cells suppress (i.e., interact). From combination experiments employing BP with CO FL, we are convinced that BP cells suppress and CO cells augment T cell proliferation of a standard MLR. That is, BP induces a functional reversal from augmentation to suppression which involves Lyt 1 cells and macrophages, probably in combination with Lyt 2 cells. The data also point to participation of cells expressing T4 since these cells were detected in abundant quantities in the FL. Isolation experiments by panning or FACS should definitevly characterize the functional nature of the T4 cells in the FL. Furthermore, from cytotoxicity and immunofluorescence data we have not only detected T4 expressing cells in the FL but have also detected unusual cell populations including T4+1-, T4-1+, and double positives. Purification experiments will help to further characterize the functional role, if any, these cells play in the FL. Therefore, the complexity of the system prompts us to direct our attention to further isolation and characterization of the components (cellular and humoral) of the fetal liver.

The data obtained in the thymus appear to have accomplished our goal, namely the functional characteristics as measured by the mixed lymphocyte response of this central organ of the immune system and the effects of BP. The increased appearance of T4+1-, T4+2- and 1-2- cells in this organ, concommitant with enhanced thymic MLR during gestation and the depression of the MLR postnatally, directs our research to more fundamental questions, namely the differentiation

pathway of T lymphocytes. Thus, does the pathway involve intermediate double positive cells or can this avenue be bypassed? And in a more applicable, but yet still fundamental vein, Does the appearance of excesses of the unusual cells detected signal a differentiation pathway that is disrupted toward accumulation of ineffective cells and/or cells that shutdown the system which are released to the peripheral lymphoid organs? Do the unusual excesses in 1-2- cells represent in situ accumulation or emigration from the fetal liver?

Although we have shown disrupted MLR in maternal thymus under the influence of BP, which undoubtedly has significance on the maintenance of an adaptable maternal immune system, judgements on the influence on the progeny must await further experimentation. Since the research points made above needed to be addressed, we were unable to provide analyses for maternal-fetal cell migrations which can be very significant in the maintenance of progeny immune status. In BP-exposed mice, the research direction taken further diluted manpower to address maternal-fetal associations in depth, and more detailed analyses of NK and CTL activity.

D. NAMES OF PERSONNEL CONTRIBUTING TO THE PROJECT.

Dr. Paul Urso, Ph.D., Principal Investigator.
Dr. Nicholas M. Ponzio, Ph.D., Consultant.
Mr. Ray A. Johnson, B.S., Research Assistant.
Mrs. Mary K. Kramer, B.S., Research assistant.
Mr. Jeffrey R. Cobb, B.S., Research Assistant (9-10 mo part-time).
Mr. Jeffrey R. Gilmour, B.S., Laboratory Technician.
Mr. Mark C. Ryan, Junior Morehouse College Undergraduate (part-time).
Ms. Doriscine Colley, Junior Morehouse School of Medicine Student (part-time).

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F. MANUSCRIPTS IN PREPARATION.

See references numbers 6, 10, 11, 12, and 17 above.