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Cytokine Responses During Mycobacterial and Schistosomal Antigen-Induced Pulmonary Granuloma Formation

Production of Th1 and Th2 Cytokines and Relative Contribution of Tumor Necrosis Factor

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Synchronized pulmonary granulomas (GRs) were induced in presensitized mice by intravenous embolization of polymer beads bound with purified protein derivative (PPD) of* Mycobacteria tuberculosis *or soluble antigens derived from* Schistosoma mansoni *eggs (SEA). Uncoated beads served as a foreign body control (CON). Antigen-coated beads elicited GRs with characteristic epithelioid macrophages and multinucleate giant cells by 4 days after embolization. Unlike PPD GR, SEA bead lesions contained eosinophils, whereas CON beads elicited only a limited mononuclear infiltrate. GRs and draining lymph nodes (LN) were assessed on days 2, 4, and 8 for Th1- (interleukin-2 [IL-2], interferon-γ [IFN]) and Th2-type (IL-4, IL-5, and IL-10) cytokines. CON GR produced only a small amount of IFN-γ on day 2 and failed to induce a significant response in draining LN. In contrast, both PPD and SEA antigen-coated beads induced reactive lymphoid hyperplasia but differed greatly in local and regional cytokine profiles. PPD GR produced IFN-γ on day 2 and the draining LN produced predominantly Th1 cytokines on days 2 and 4. In contrast, SEA bead GRs were dominated by Th2 cytokines. The corresponding LN produced IL-2 and IL-4 on day 2; IL-2, IL-4, IFN-γ, and IL-10 on day 4; then IL-2, IFN-γ, and IL-4 on day 8, probably reflecting maturational changes of T cells. Macrophages (MP) from bead GR also showed different patterns of IL-6 and tumor necrosis factor (TNF) production. Compared with CON GR, MPs from PPD GR were weak sources of IL-6, whereas those of SEA GR showed enhanced and accelerated production. In contrast, MP of PPD GR had augmented TNF-producing capacity, whereas those of SEA GR showed delayed TNF production.* In vivo *depletion of TNF, respectively, caused 40 and 10% decreases in PPD GR and SEA GR but had no effect on CON GR area, indicating that TNF contributed to a greater degree to the PPD response. These data show that depending on the inciting agent, GR can be mediated by different cytokines. Characterization of inflammatory lesions by cytokine profiles should allow design of more rational therapeutic interventions. (Am J Pathol 1994, 145:1105–1113)

Granulomas (GRs) are a form of chronic inflammation found in a wide variety of diseases.[1] These lesions can differ significantly in histological appearance but have a consistent element of mononuclear phagocytes and their derivatives, epithelioid macrophages, and multinucleate giant cells. Warren offered a classification of GR based on the degree of antigen-specific immune involvement and cellular turnover that has been useful in approaching the study of these lesions.[2] Based on this classification, GR associated with infectious agents such as *Mycobacteria* species, fungi, and helminths are considered hypersensitivity

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type, whereas inert poorly digestible particles induce nonimmune foreign body-type lesions.

Information derived from a variety of experimental models suggests that a number of cytokines play a role in GR formation but it has not revealed a common underlying mechanism. For example, *in vivo* studies of mycobacterial GR indicated that interferon-γ (IFN-γ) and tumor necrosis factor (TNF) were critical cytokines,[3,4] whereas studies of the *Schistosoma mansoni* egg GR indicated that interleukins-2 (IL-2) and -4 dictate the degree and quality of the inflammation.[5–8] These cytokine profiles suggested that hypersensitivity-type granulomatous inflammation involved both Th1 and Th2 cells. Because of the disparate nature of the above mentioned models, it is difficult to directly compare immunopathological events in a systematic manner.

This study was undertaken to establish models of Th1 and Th2 pulmonary granulomatous inflammation that would allow for detailed analysis and manipulation. This was accomplished by presensitization of mice with *Mycobacteria* species (BCG strain) or *Schistosoma mansoni* eggs followed by pulmonary embolization of carbohydrate beads coated with a known quantity of soluble antigens derived from the respective organisms. Analysis of cytokines produced by the isolated GR and draining lymphoid tissue indicate that different cytokines are involved in the generation of these lesions. Moreover, granulomatous inflammation can be characterized by the pattern of cytokine involvement that probably reflects evolutionary adaptations to various environmental pathogens.

Materials and Methods

Animals and Schistosome Egg Isolation

Female CBA/J (The Jackson Laboratories, Bar Harbor, ME) mice were used in all experiments. Mice were maintained under specific pathogen-free conditions and provided with food and water ad libitum. Schistosome eggs were isolated aseptically from the livers of Swiss albino mice infected with 200 to 300 cercariae of the Puerto Rican strain of *Schistosoma mansoni*, as previously described.[9]

Sensitization and GR Induction

Mice were sensitized by intraperiteneal injection of 4 mg (wet weight) of live Bacille-Calmette-Guerin (BCG) strain of *M. bovis* (Organon Technika, Durham, NC) or 3000 *S. mansoni* eggs suspended in 0.5 m of phosphate-buffered saline (PBS). Fourteen to 16 days later BCG- and egg-sensitized mice were, respectively, challenged by tail vein with 6000 Sepharose 4B beads (in 0.5 ml PBS) coated with purified protein derivative (PPD) of *M. tuberculosis* (kindly provided by Dr. J. Cornecelli, Warner Lambert/Parke Davis, Ann Arbor, MI) or soluble schistosome egg antigens (SEA) obtained from the World Health Organization, Geneva, Switzerland. Uncoated beads were administered to control animals. Beads embolized to the lungs elicited GR that were examined at 2, 4, and 8 days after injection, as described below.

Preparation of Antigen-Coated Beads

Cyanogen bromide-activated Sepharose 4B beads (Sigma, St. Louis, MO) were swollen and coupled with PPD or SEA as previously described.[10] Briefly, 1 ml of swollen beads was mixed with 8 mg of antigens dissolved in 2 ml coupling buffer (0.1 M $NaHCO_3$, 0.5 M NaCl) and agitated on a rotary mixer overnight at 4 C. Remaining protein was measured in the bead supernate to determine coupling efficiency. Total protein coupled was determined to be 4 ng/bead. Control beads were blocked with ethanolamine. Beads were stored at 4 C in sterile PBS with 0.05% NaN_3. Beads were washed three times in preservative-free, sterile PBS and counted before injection.

GR, GR Macrophage, and Lymph Node (LN) Culture

Groups of mice were killed on 2, 4, and 8 days after bead embolization. After perfusion with cold RPMI, lungs, excluding the trachea and major bronchi, were excised then placed in cold RPMI medium and homogenized in a Waring blender with a narrow-bottom stainless steel cup. GRs were collected over a sterile stainless steel mesh (no. 100) and rinsed with cold RPMI. Intact lesions were suspended to 1500/ml of RPMI medium containing 10% fetal bovine serum (FBS), 100 mmol/L L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (RPMI-FBS) then cultured in the presence or absence of 5 μg/ml PPD or SEA at 37 C in a 5% CO_2 humidified atmosphere. Supernatants were collected by centrifugation at 24 and 48 hours and frozen at −80 C.

GR macrophages were obtained by digestion of intact GR in a membrane-sterilized solution of RPMI-FBS containing 1000 U/ml type IV collagenase (Sigma). After a 30-minute incubation in a 37 C shaker water bath at 120 cycles/minute, the digest was passed through a stainless steel mesh (no. 100) and washed four times in RPMI-FBS. Macrophages were

isolated by 2-hour adherence and cultured on 35-mm plastic dishes as above in RPMI-FBS in the presence or absence of 1 μg/ml lipopolysaccharide (LPS) (Sigma; *Escherichia coli* O111 type). Supernatants were collected at 48 hours and stored as above. Adherent cells on dishes were stained and the total number of adherent macrophages was counted to normalize cytokine production. Adherent cells were >90% macrophages based on morphology and nonspecific esterase staining.

Mediastinal LNs were collected at the time of lung harvest and teased into a single cell suspension. After washing, the cells were cultured in RPMI FBS a 5×10^6/ml in the presence or absence of 5 μg/ml PPD or SEA then cultured as above for 36 hours. To determine whether there was any direct cytokine induction by antigens, control (CON) cultures were stimulated with both PPD and SEA. Supernatants were collected by centrifugation and stored at −80 C.

In Vivo *Cytokine Depletion*

At the time of bead challenge mice were given an intraperitoneal injection of 5 mg of IgG prepared by protein A column purification of rabbit anti-mTNF-α serum. The characterization of this neutralizing antibody has been previously reported.[11,12] Nonimmune rabbit IgG served as a control. Four days after challenge mice were killed and granulomatous lungs were excised, fixed in buffered formalin, and prepared for histological examination.

GR Measurement

GR area was measured blindly in hematoxylin and eosin-stained sections using a morphometer and software program (The Morphometer; Woodshole Educational Associates, Woodshole, MA). A minimum or 20 lesions were measured per lung.

Cytokine Measurement

IL-2 was measured by the standard CTLL-2 proliferation assay using the method of Tada et al.[13] in the presence of anti-IL-4 receptor and anti-IL-4 antibodies (Genzyme, Cambridge, MA) to block interference by IL-4. Recombinant murine IL-2 was used as a standard and sensitivity was 1 U/ml. IL-4 was measured by a standard assay using the CT 4S IL-4-dependent T cell clone developed by Dr. W. E. Paul,[14] and sensitivity was 0.5 U/ml.

Recombinant murine IL-4 standard was obtained from Genzyme. IL-5 was measured by ELISA using dual monoclonal antibodies TRFK-4 and TRFK-5 (ATCC, Rockville, MD), as previously described.[15] Sensitivity was 250 pg/ml and recombinant murine IL-5 (R&D, Minneapolis, MN) served as a standard. IFN-γ was measured by ELISA as described[16] using a capture antibody derived from the XMG-6 clone, kindly provided by Dr. F. Finkelman, NIH; sensitivity was to 50 pg/ml. IL-10 and TNF-α were determined by ELISA using commercially available reagents (PHARMINGEN, San Diego, CA); sensitivities were 50 and 100 pg/ml, respectively. IL-6 was measured by the standard B9 cell proliferation bioassay, as previously described;[17] sensitivity was 10 pg/ml. Commercially available recombinant murine cytokines served as standards in all assays (Genzyme and Preprotech Inc., Rocky Hill, NJ).

Statistics

The Student's *t*-test was used to compare groups. Values of $P > 0.05$ were considered to indicate lack of significance.

Results

Histology of Bead GR Formation

As shown in Figure 1, GR elicited by PPD, SEA, and CON beads had distinctive histological characteristics. At 2 days, all beads contained a rim of polymorphonuclear leukocytes surrounded by an infiltrate of mononuclear cells. A few infiltrating eosinophils were also present in SEA GR. By 4 days, PPD GR were composed almost entirely of small and large mononuclear cells, whereas SEA GR also contained a significant component of eosinophils. CON GR were consistently smaller than those of antigen-coated beads and by 4 days were composed of a modest infiltrate of mononuclear cells. Multinucleate giant cells could be found in all three bead lesion types. By 8 days, all of the lesions had diminished in size but SEA bead GR tended to be larger, persisted longer, and were associated with perivascular stromal proliferation reminiscent of the "pipestem" fibrosis associated with hepatosplenic schistosomiasis (data not shown).

Kinetics of Bead GR Formation and Regional Lymphoid Proliferation

Figure 2 shows the mean cross-sectional area of bead GR and yield of lymphocytes obtained from the mediastinal LN (hilar and paratracheal) during the 8-day study period. Both PPD and SEA bead GR achieved maximal size on day 4 and diminished thereafter. The CON beads elicited a consistently

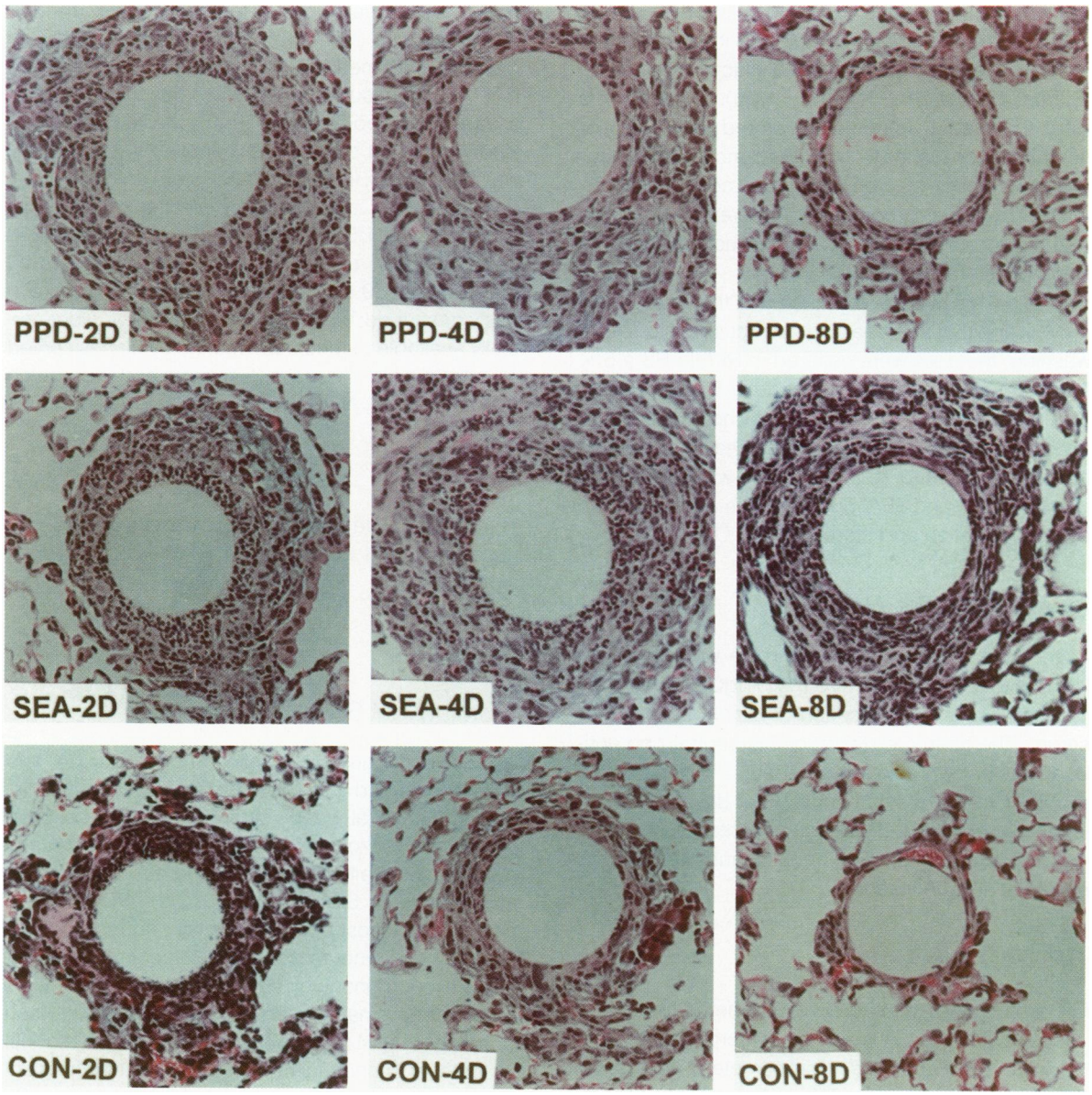

Figure 1. *Histological appearance of synchronized pulmonary GR formation in response to PPD, SEA, and CON beads. Beads coated with PPD of* M. tuberculosis, *soluble SEA, or no antigens (CON) were embolized by intravenous injection in separate groups of mice, lungs were removed at 2, 4, and 8 days then examined in hematoxylin and eosin-stained sections. Magnification × 100.*

smaller response but it was maximal on day 4. In the LN, PPD and SEA beads elicited a comparable lymphoid proliferation that was maximal on day 4. In contrast, CON beads did not stimulate a significant LN response.

Cytokine Profiles of Cultured Bead GRs and Regional LNs

Intact pulmonary bead GRs were isolated and cultured at 2, 4, and 8 days of development. PPD and SEA bead GR were cultured in the presence of 5 μg/ml PPD or SEA, respectively. CON bead GR were cultured in the presence of both PPD and SEA. As shown in Figure 3, supernatants of cultured lesions had clearly different cytokine profiles. Of five cytokines measured (IFN-γ, IL-2, IL-4, IL-5, and IL-10) only IFN-γ was detectable at 2 days in PPD and CON bead cultures. In contrast, the SEA-GR produced only small amounts of IFN-γ but significant amounts of IL-4 were present on days 2 and 4 and IL-5 was detected at all of the assay points reaching maximum levels on day 4. Notably, both IL-2 and IL-10 levels were generally trace to undetectable in all GR cultures.

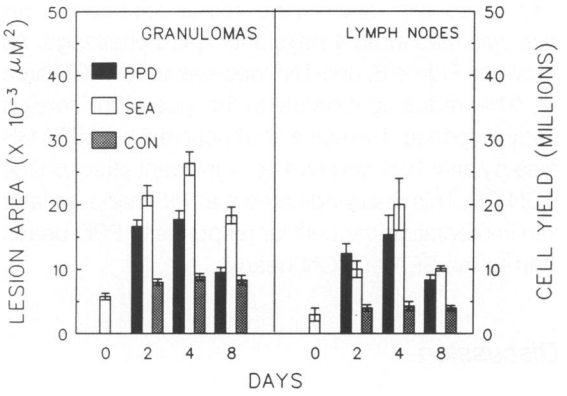

Figure 2. *Kinetics of pulmonary GR formation and the regional LN response to challenge with PPD, SEA, and CON beads. Bars are means ± SEM of values derived from 9 to 12 mice. Only a single bar is shown at time 0 because no differences were observed between groups at this time.*

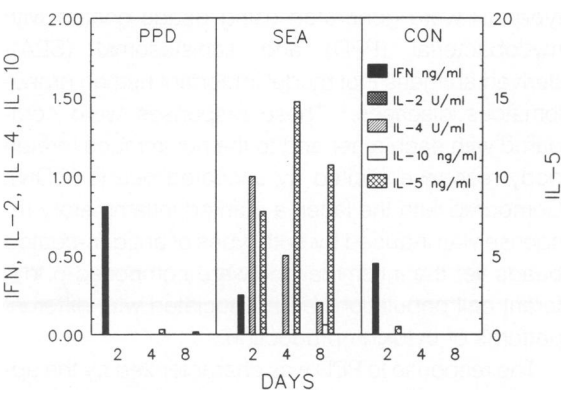

Figure 3. *Cytokine profiles of intact, cultured synchronously developing bead GRs. Bead GRs were isolated and cultured in the presence of specific antigens as described in Materials and Methods. Supernatants were collected and assayed for IFN-γ, IL-2, IL-4, IL-5, and IL-10. Bars show antigen-elicited levels of cytokines and are means of three separate experiments. Standard errors were less than 10% of means.*

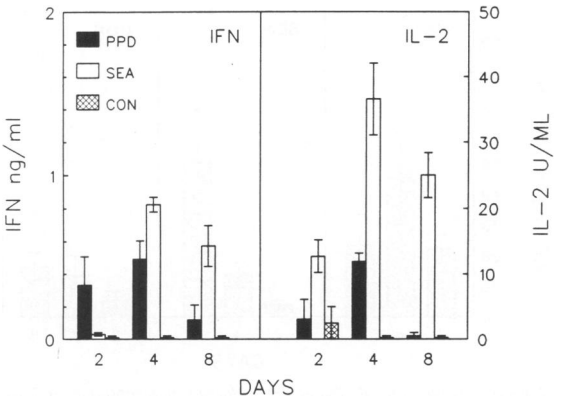

Figure 4. *Production of IFN-γ and IL-2 by cultured draining lymphoid tissue during synchronous bead GR formation. Bars show antigen-elicited levels of cytokines and are means ± SEM of three to four separate experiments with a total of 9 to 12 mice per group.*

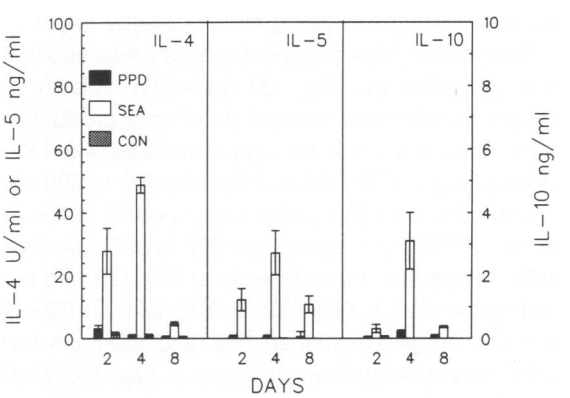

Figure 5. *Production of IL-4, -5, and -10 by cultured draining lymphoid tissue during synchronous bead GR formation. Bars show antigen-elicited levels of cytokines and are means ± SEM of three to four separate experiments with a total of 9 to 12 mice per group.*

LN cultures likewise had distinct patterns of cytokine production. Figure 4 shows the pattern of Th1-associated cytokines, IFN-γ and IL-2, produced by LN during the course of GR formation. IFN-γ and IL-2 were detected in the LN during formation of both PPD and SEA bead lesions but in the PPD group IFN-γ and IL-2 were produced on days 2 and 4, whereas in the SEA group these cytokines predominated on days 4 and 8. The LN of CON bead-injected mice produced only small amounts of IL-2 on day 2 (≤2 U/ml). As with GR cultures, levels of Th2 cytokines clearly distinguished the PPD and SEA response. As shown in Figure 5, IL-4, IL-5, and IL-10 were detected primarily during SEA bead GR formation. IL-4 dominated on days 2 and 4 then dropped off by day 8. Both IL-5 and IL-10 levels were greatest on day 4.

To summarize, these results suggested that Th1-related cytokines mediated PPD bead GR formation and Th2-related cytokines mediated SEA bead GR formation. The CON bead elicited a weak nonspecific reaction associated with low transient levels of local IFN-γ production and no significant regional lymphoid response.

GR Macrophage TNF and IL-6 Production

To determine the potential contribution of monokines to GR formation, macrophages isolated from synchronously developing bead GR were assessed for their capacity to produce TNF and IL-6. As shown in Figure 6, macrophages from bead GR displayed distinct patterns of spontaneous TNF production as the lesions matured. PPD GR macrophages showed an early wave of augmented TNF production that was maximum on day 4 (45 ± 15 ng/million). SEA GR macrophages showed a delayed onset of TNF production that was maximum on day 8 (50 ± 15 ng/million). CON

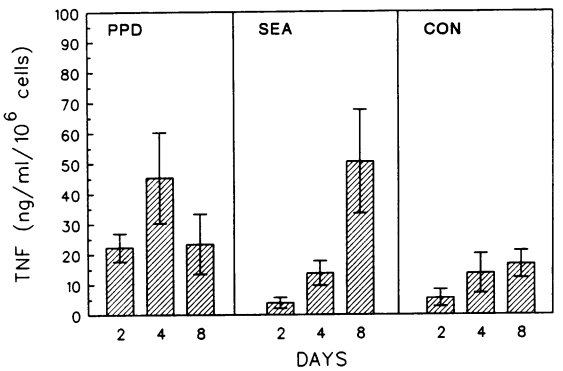

Figure 6. *Production of TNF-α by cultured macrophages isolated from synchronously developing bead GRs. Bars show spontaneously released levels of TNF and are means ± SEM of three to four separate experiments with a total of 9 to 12 mice per group.*

macrophages showed the least TNF production, achieving only 10 to 20 ng/million cells by day 8.

As shown in Figure 7, spontaneously released IL-6 was generally less than 20 ng/million cells in all groups but distinctly different patterns of production were noted when cultures were stimulated with LPS. Macrophages of CON GR produced 400 to 500 ng/million cells over the 8-day study period, whereas those of PPD lesions produced 200 to 300 ng/million cells. In contrast, macrophages of SEA GR had notably enhanced IL-6-producing capacity on days 2 and 4 (averaging more than 1400 and 800 ng/million cells, respectively) then dropped to near CON GR levels by day 8. Thus, macrophages from SEA GR displayed an inverse relationship between TNF- and IL-6-producing capacity.

Effect of In Vivo *TNF Depletion*

To test the relative participation of TNF in the three types of GR, lesions were induced in mice passively

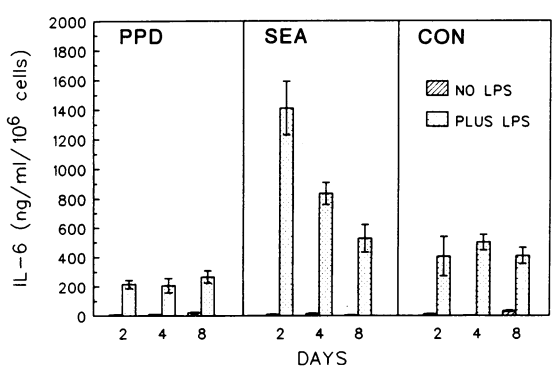

Figure 7. *Production of IL-6 by cultured macrophages isolated from synchronously developing bead GRs. Bars show spontaneously released and LPS-elicited levels of IL-6 and are means ± SEM of three to four separate experiments with a total of 9 to 12 mice per group.*

immunized with anti-murine TNF-α antibodies. GR size was measured 4 days after bead challenge. As shown in Figure 8, anti-TNF decreased PPD GR area by 40%, reducing it nearly to the size of the foreign body response. The same treatment reduced SEA GR area by only 10% and had no significant effect on the CON GR. This result indicated that TNF had a greater role in mediating the cellular response to PPD beads than to the SEA or CON beads.

Discussion

Our study provides a detailed definition of models of synchronous pulmonary granulomatous inflammation and reveals important information regarding the participation of selected cytokines in responses to different antigenic stimuli. Two forms of hypersensitivity-type GR were generated using beads coated with mycobacterial (PPD) and schistosomal (SEA)-derived antigens that model important human granulomatous diseases.[1] These responses were compared with each other and to the nonimmune foreign body response elicited by uncoated beads (CON). Compared with the latter, a marked inflammatory response was induced by both types of antigen-coated beads yet the inflammations were composed of different cell populations and associated with different patterns of cytokine production.

The response to PPD was characterized by the appearance of IL-2 and IFN-γ in the LN and primarily IFN-γ at the site of inflammation. This pattern is consistent with previous reports that Th1 cells mediate the response to mycobacterial infections.[18,19] In the LN, SEA beads also induced IL-2- and IFN-γ-producing cells but in addition there was prominent

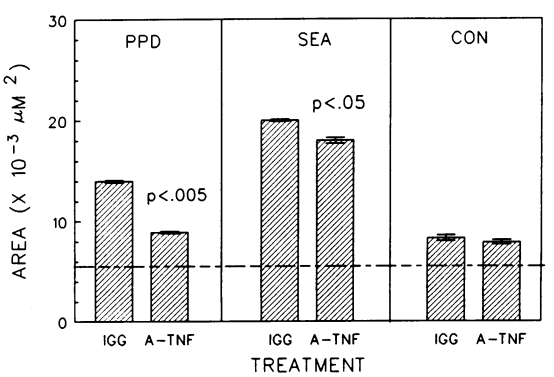

Figure 8. *Effect of anti-TNF-α treatment on synchronously developing PPD, SEA, and CON bead GRs. At the time of bead challenge, mice (6/group) were given 5 mg of control IgG (IGG) or anti-TNF IgG (A-TNF) by intraperitoneal route. Four days later, mice were sacrificed and lungs were removed for morphometric evaluation. Bars are mean GR area ± SEM; a minimum of 20 lesions were measured from each lung. Dashed line indicates mean cross-sectional area of beads only.*

production of IL-4, IL-5, and IL-10. The local SEA bead granulomatous response was dominated by Th2 cytokines IL-4 and IL-5 and low levels of IL-10. Comparatively, IFN-γ was a minor component of the SEA bead GR and IL-2 was not present or at levels below assay sensitivity. This pattern of cytokine expression is similar to that previously reported for the schistosome egg GR by Grzych et al,[7] Henderson et al,[20] and Chensue et al.[8] Previously, Th1 cells were thought to mediate delayed-type hypersensitivity, whereas Th2 cells were thought to primarily promote antibody production[21–23] but there is growing evidence that Th2-associated cytokines IL-4 and IL-5 are critical in some forms of inflammation.[24,25] For example, Sher et al[26] provided direct evidence that IL-5 is largely responsible for eosinophil recruitment during *S. mansoni* infection. In accord with these findings, we showed that IL-5 was produced by the eosinophil-rich SEA GR. It is not known whether IL-4/IL-5-evoked IgE production is also involved in Th2-related inflammation.[26] Nevertheless, our studies provide evidence that both Th1- and Th2-associated cytokines participate in cellular inflammatory responses.

It is not clear to what degree Th1 and Th2 cytokines collaborate in granulomatous responses, but several studies suggest that there is a significant degree of antagonistic cross-regulation.[27] Depletion studies using the bead models described herein indicate that neutralization of IFN-γ significantly exacerbates the SEA lesion but abrogates the PPD bead GR (manuscript in preparation). This finding suggests that Th1 and Th2 cytokines can mediate cellular infiltration independently but it remains to be determined what qualities of the lesions are affected by these treatments and if there is temporal participation of Th1 and Th2 cells. We have previously shown that schistosome eggs evoke an initial Th1 cytokine profile in the primary response followed by a dominant Th2 profile in the secondary response.[28] Likewise, an elegant study reported by Orme et al[29] indicated that Th1 and Th2 cells participate sequentially in the response to *Mycobacteria* and suggests that the Th2 phase is related to lowered resistance. Unlike those workers we did not detect Th2-associated cytokines during PPD bead GR formation, but this may be because we challenged sensitized mice before the onset of the Th2 response. Future studies will need to establish whether PPD beads evoke a Th2 response late (>20 days) after BCG sensitization.

The observed lack of expression of IL-2 by GR was previously reported for the response to schistosome eggs[20,28] and now appears to apply to the PPD response. Although it is possible that IL-2 was rapidly sequestered to receptors on GR cells, our finding might indicate that IL-2 participates to a lesser degree within GR. Our findings support the prevailing concept that Th1 and Th2 cells lose or reduce their IL-2-producing capacity as they become effector cells.[30] This principle may also apply to IL-10, which likewise showed disparate local and regional expression.

The LN response to CON beads was negligible and only IFN-γ was detected at the GR. Both the CON and PPD bead GR transiently produced IFN-γ on day 2, whereas IFN-γ was a minor element in the SEA lesions. Although PPD lesions produced greater amounts than CON lesions, it is not clear whether this is due to the larger size of the lesion or greater T cell participation. Presumably, antigen-specific T cells are not involved in the response to CON beads, hence the IFN-γ produced by GR may be derived from other cell populations such as natural killer cells.[31] This notion is fully compatible with the finding that natural killer cells are a significant component of the lymphocyte population recruited to foreign body bead GR.[32]

An important aspect of our study was the assessment of GR macrophage, TNF, and IL-6 production. Both PPD and SEA bead GR macrophages showed enhanced TNF-producing capacity compared with CON lesions, and the time course of production was in agreement with that described previously for schistosome egg and foreign body-type GR.[33] Macrophage activating cytokines are probably enhancing TNF-producing capacity in the hypersensitivity lesions. Because IFN-γ is known to augment TNF production *in vitro*,[34] it is potentially an important activating agent in the PPD lesion. The activating agent in SEA GR is more problematic and has yet to be elucidated. Moreover, TNF appeared to be down-regulated in the early phase of SEA GR formation. It was noted that macrophages of SEA GR produced less TNF than those of PPD GR on days 2 and 4 of development, coinciding with the time of maximal IL-4 production. In view of reports that Th2 cell products can down-regulate TNF production,[35,36] it is tempting to speculate that these factors are responsible for the delayed TNF response of SEA GR.

The differing patterns of IL-6 production were likewise intriguing. The macrophages of Th1 lesions had diminished whereas those of Th2 lesions had enhanced IL-6-producing capacity. Because IL-6 is probably important to humoral responses, our findings suggest that macrophages of Th2 but not Th1 lesions are primed to promote antibody production. The latter also seems to reflect the lesser role of humoral immunity in resistance to *Mycobacteria* species, suggesting a teleological basis for the observed

differences. Surprisingly, macrophages of Th2 lesions displayed an inverse relationship between TNF and IL-6 production, implying coordinated expression and/or differential regulation of these cytokines.

Our *in vivo* depletion study provides further support to the notion that TNF plays an important role in the response to *Mycobacteria* species.[3,4] However, TNF appeared to play a lesser role in the secondary response to schistosomal antigens. On face value, this result seems to conflict with the findings of Amiri et al,[37] suggesting that TNF was a critical mediator of schistosome egg GR formation. However, those workers reconstituted inflammation with exogenous TNF in immunodeficient mice and they did not examine the histological character of the lesions they induced. We suggest that they generated a Th1-like granulomatous response to schistosome eggs and not the Th2-like response elicited during a secondary response in immunologically intact mice. We recently reported that TNF is important to the primary response to schistosome eggs,[38] which has a Th1-like profile.[28] Thus, TNF seems to have a greater role in the primary response and is probably down-regulated during the secondary response by cytokines such as IL-4 and IL-10.[35,36]

Our findings indicate that hypersensitivity-type granulomatous inflammation represents heterogeneous responses dictated by different cytokines. This heterogeneity probably arose as the immune system was forced to adapt to the appearance of different environmental pathogens and may reflect programmed responses to specific antigens. For example, mycobacterial antigens can act as direct stimuli for γ/δ T cell proliferation,[39] and these cells may contribute strongly to the PPD response. Regardless of the cellular source of cytokines, we propose that GR be classified according to their pattern of cytokine involvement. This study suggests that immune-type GR can at least be classified into IFN/TNF (Th1-like) dominant and IL-4/IL-5 dominant (Th2-like) lesions. This approach will allow therapeutic interventions to target cytokines critical to each class of inflammation. As more detailed analyses are performed further categories may become apparent and other mediators may serve to better classify inflammatory lesions.

Acknowledgment

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