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Temporal Relationship of Cytokine Release by Peripheral Blood Mononuclear Cells Stimulated by the Streptococcal Superantigen pep M5

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We undertook this study to determine the quality, quantity, and temporal relationship of pep M5-induced cytokine release. The ability of pep M5 to stimulate interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) production by a T-cell-depleted, monocyte- and B-cell-enriched cell population was dependent on the presence of T cells. The requirement for T cells could be met by addition of exogenous gamma interferon (IFN-γ). In the presence of IFN-γ, pep M5 induced the release of TNF-α, IL-1, and IL-6. TNF-α levels peaked at 24 h, while IL-1 and IL-6 levels peaked at 48 h. pep M5 induced T cells to produce IFN-γ, which may have accounted for the ability of the superantigen to induce the production of IL-1, IL-6, TNF-α, and TNF-β by peripheral blood mononuclear cells (PBMC). The addition of excess IFN-γ to cultures of pep M5 and PBMC did not further increase the release of these cytokines at 24 and 48 h but resulted in sustained higher levels at 72 h. Interestingly, TNF-β production occurred only in the presence of pep M5 and exogenous IFN-γ. The ability of pep M5 to induce cytokine production was compared with that of a potent superantigen, staphylococcal enterotoxin B (SEB). SEB was a 2- to 14-fold-more-potent inducer of IFN-γ production. Furthermore, the profile of cytokine released by PBMC in response to this superantigen mimicked that seen with pep M5 in the presence of exogenous IFN-γ. In conclusion, pep M5 induces the production of cytokines that are involved in immune regulation and inflammation. These cytokines also play a major role in human T-cell responses to this superantigen.

Bacterial infections may develop into potentially fatal illnesses such as toxic shock and autoimmunity. The mechanism of pathogenesis of these diseases is not always clear, but it is well established that both gram-positive and gram-negative bacteria produce virulence factors that are powerful stimulators of host immune responses and potent inducers of inflammatory cytokines, which are believed to have an important role in mediating the pathogenesis of infectious diseases and their nonsuppurative sequelae (47).

Group A streptococci can induce a variety of acute infections ranging from pharyngitis to streptococcal toxic shock syndrome (42–46). In addition, 3 to 10% of infected individuals may develop poststreptococcal autoimmune diseases which can target the heart, kidney, brain, or skin (3, 46). Group A streptococci produce a number of potential virulence factors such as streptolysin O, streptolysin S, pyrogenic toxins A through C, adhesins, immunoglobulin-binding proteins, and a variety of enzymes, including proteases, DNase, and peptidases. A number of these factors are known to cause disease. For example, the pyrogenic toxins are associated with scarlet fever and streptococcal toxic shock syndrome (7, 41–44). However, a major virulence factor of group A streptococci is the surface M protein, which allows the bacteria to evade phagocytosis by binding to host serum factors (15, 53). There are more than 80 different M protein serotypes. The ones that have been characterized are homologous at the carboxy-terminal half of the molecule but highly variable at the amino-terminal end, which harbors the type-specific epitopes (2, 15). M protein has been implicated in the pathogenesis of rheumatic fever, rheumatic heart disease, and glomerulonephritis (3, 45). Of the 80 different serotypes, only 11 or 12 serotypes show a high degree of association with rheumatic fever and are therefore called rheumatogenic serotypes (45). This group includes organisms of M serotype 5, which until the 1980s was the serotype most commonly associated with the disease (42, 52).

Recently, we showed that pepsin-purified type 5 M protein (pep M5) is a superantigen that specifically stimulates human T cells expressing Vβ2, Vβ4, and Vβ8 elements (48, 50) and that the Vβ specificity of pep M5 is quite distinct from those of the pyrogenic exotoxins (49). Although pep M5 stimulates a powerful immune response, it is less potent than other superantigenic toxins such as staphylococcus enterotoxin B (SEB) and streptococcal pyrogenic enterotoxins A and C (SPEA and SPEC) (49). This reduced potency of pep M5, which may turn out to be important for its role in autoimmunity, could be related to the quantity, quality, and temporal relationship of cytokine release. To further understand the interaction between this streptococcal superantigen and the human immune system, we undertook the present study to determine the ability of pep M5 to induce the production of inflammatory cytokines, namely, interleukin-1 (IL-1), IL-6, tumor necrosis factor alpha (TNF-α), TNF-β, and gamma interferon (IFN-γ). The effects of pep M5 are compared with those of a potent superantigen, SEB.

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MATERIALS AND METHODS

Purification of M protein. M protein was purified by limited pepsin digestion of type M5 group A streptococci (2). The purified fragment, designated pep M5, was further purified by high-pressure liquid chromatography (HPLC) DEAE (Protein PAK 5 PW) anion-exchange chromatography. The 60% ammonium sulfate pellet was dissolved and dialyzed against phosphate-buffered saline and then applied to the HPLC column. The protein was eluted with a gradient of 0 to 0.5 M sodium sulfate in 20 mM Tris SO_4 buffer, pH 7.8. The fractions were analyzed by electrophoresis and migrated as a single band in silver-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Western immunoblots were performed by using antibodies to synthetic peptides of the M5 protein to confirm that the single band on the gel was indeed pep M5.

Although it has recently been claimed that the mitogenic activities of M proteins are due to contamination with pyrogenic toxins (15a), there is ample evidence that our preparation is free of these toxins. First, we recently demonstrated that the pattern of Vβ specificity of pep M5 is quite distinct from those of the pyrogenic exotoxins (49). pep M5 stimulates T cells bearing Vβ2, Vβ4, and Vβ8; SPEA stimulates Vβ2-, Vβ12-, Vβ14-, and Vβ15-bearing T cells; SPEB is specific for Vβ8; and SPEC is specific for Vβ1, Vβ2, Vβ5.1, and Vβ10. If pep M5 was contaminated with SPEA, we would have seen expansion of Vβ12 and Vβ14 in addition to Vβ2, Vβ4, and Vβ8, which are specific for pep M5. If the pep M5 was contaminated with SPEC, we would have seen evidence for expansion of Vβ1, Vβ5.1, and Vβ10, and we did not. Similarly, if the pep M5 activity was due to SPEB, we would have seen expansion of only Vβ8. Finally, if pep M5 was contaminated with all three toxins, we should see expansion of Vβ1, -2, -4, -5.1, -8, -10, -12, -14, and in certain cases -15. Earlier studies by Beachey et al. (1b) showed that pepsin-extracted M proteins were devoid of toxin activity by showing that injection of up to 40 μg of pep M preparations failed to produce a positive skin test. Second, unlike the effects of pyrogenic toxins, the mitogenic effect of the pep M protein is specific to human T cells. In 1981, Dale et al. (7a) reported that none of the lymphocytes from unimmunized laboratory animals, including mice and rabbits, responded to in vitro stimulation with pep M5, pep M6, or pep M24. In their study, the mitogenic activity of the M protein was tested at 10 and 50 μg per culture, which are 50 and 250 μg/ml. In our laboratory, mouse T cells stimulated with 10, 30, and 100 μg of pep M5 per ml failed to proliferate (31a). The same preparation of pep M5 stimulated human T cells at 0.5 ng/ml. Third, we have recent evidence that other rheumatogenic serotypes of pep M proteins are also superantigens and that each has a unique pattern of Vβ specificity, suggesting that the M proteins represent a family of structurally related streptococcal superantigens (52a). Finally, researchers in England have made recombinant M5 protein and found that it stimulates brisk blastogenic responses in human but not mouse T cells (39a). These issues have been recently reviewed (31).

PBMC purification. Peripheral blood was obtained from healthy adults, and peripheral blood mononuclear cells (PBMC) were isolated by the Ficoll-Hypaque method (4). T cells were separated from monocytes and B cells by subjecting freshly isolated PBMC to two cycles of erythrocyte rosetting (27) at 37°C. The E-rosette-negative population enriched in B cells and monocytes was used as antigen-presenting cells (APC). PBMC or purified APC were seeded at 10^6 cells per ml in 24-well plates and cultured in RPMI medium containing 10% heat-inactivated fetal bovine serum and supplemented with 4 mM L-glutamine, 100 U of penicillin G per ml, and 100 μg of streptomycin per ml (RPMI complete medium). The cells were incubated for the indicated times, and then the culture media were collected and centrifuged for 8 min at 400 × *g* to remove contaminating cells. The culture supernatants were aliquoted and stored at −20°C until further analysis for cytokine activity.

Cytokine assays. Mononuclear cell supernatants were assayed in duplicate for TNF-α, IL-6, IL-1β, and TNF-β by using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, Minn.). The assays for IFN-γ and some assays for IL-6 activity were conducted by using standard assay EIA Test Kits from Amgen Diagnostics (Thousand Oaks, Calif.). The assays were performed according to the manufacture's instructions, and the data were calculated from standard curves prepared for each run. IL-1 activity in the culture supernatants was assessed by the method of Kaye et al. (29) using the mouse T-helper-cell clone (D10 G4.1) cultured in the presence of 1 μg of phytohemagglutinin A per ml. Units of IL-1 activity were calculated from cultures of T-helper-cell clone containing recombinant IL-1 as the standard.

RESULTS

Effect of pep M5 on production of cytokines by T-cell-depleted APC. First, we asked whether pep M5 can directly induce the production of cytokines by APC. Populations of APC depleted of T cells and enriched in monocytes and B cells were cultured with pep M5, and the levels of IL-1, IL-6, and TNF-α in the culture supernatant were measured by ELISA at various times after the initiation of culture. As shown in Fig. 1, pep M5 activated the APC to produce low levels of IL-1 and TNF-α. Addition of IFN-γ to the culture significantly enhanced the release of both cytokines. Similarly, IFN-γ enhanced production of IL-6 by pep M5; however, in contrast to what was observed with the production of TNF-α and IL-1, measurable quantities of IL-6 were produced by pep M5 alone. We used 100 U of IFN-γ per ml, because as shown in Fig. 2, the ability of IFN-γ to augment the release of cytokines by pep M5-activated APC plateaued at this concentration.

In the presence of IFN-γ, IL-1 was detected as early as 24 h, peaked at 48 h, and remained constant at 72 h. In contrast, although IL-6 levels in the presence of IFN-γ reached a peak at 48 h, they dropped dramatically by 72 h. The peak rise in TNF-α levels, which took place only in the presence of IFN-γ, occurred at 24 h and then continued to decline until 48 and 72 h. Not surprisingly, cultures of APC and pep M5 in either the presence or absence of IFN-γ produced no detectable levels of TNF-β, which is primarily produced by T cells (data not shown).

Although levels of cytokine production varied from one individual to another, the ability of IFN-γ to augment release of the inflammatory cytokines in all individuals tested had a very similar kinetic pattern and temporal relationship (Fig. 3).

Effect of pep M5 on production of cytokines by PBMC. In contrast to APC populations, PBMC responded to pep M5 alone by producing high levels of IL-1, IL-6, and TNF-α. Addition of IFN-γ had little or no effect on the levels of these cytokines (Fig. 4). IL-1 levels were elevated in cultures of PBMC with either pep M5 or IFN-γ alone. IL-1 levels peaked at 48 h and were 8 and 6 U/ml for pep M5- and

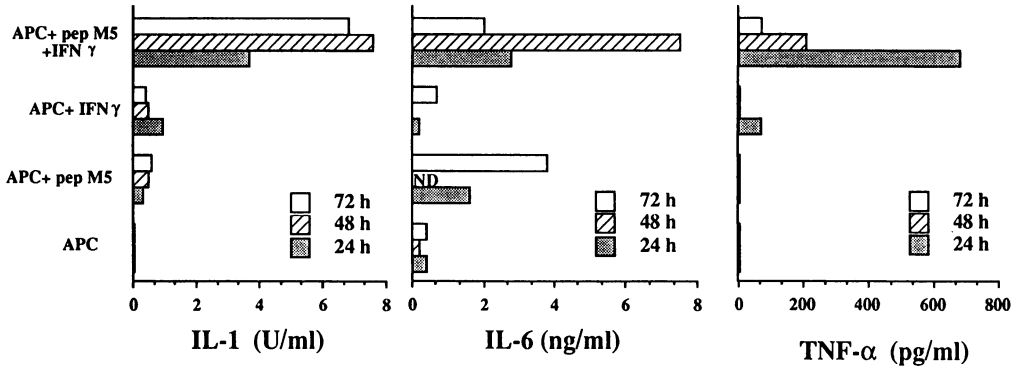

FIG. 1. Cytokine release by T-cell-depleted APC induced by pep M5 in the presence and absence of IFN-γ. APC were seeded at 10^6 cells per ml in 24-well plates and cultured in RPMI complete medium for 24, 48, or 72 h, and then the culture media were collected and centrifuged for 8 min at 400 × *g* to remove contaminating cells. As indicated, some cultures were stimulated with 1 μg of pep M5 per ml in the presence or absence of 100 U of IFN-γ per ml. Culture supernatants were aliquoted and stored at −20°C until further analysis for cytokine activity as described in Materials and Methods. Data are presented as averages of duplicate determinations and are representative of at least three different experiments. ND, not determined.

IFN-γ-containing cultures, respectively. At 72 h, IL-1 levels declined in both cultures. However, when pep M5 and IFN-γ were added together, the levels of IL-1 remained elevated at 48 h (9 U/ml), but there was no decline at 72 h; instead, IL-1 levels increased to 10 U/ml.

pep M5 alone induced the production of IL-6, which peaked at 24 h (19 U/ml) and declined gradually by 72 h to 10 U/ml. Although the addition of IFN-γ to pep M5-stimulated PBMC had little effect on IL-6 levels at 24 and 48 h, IFN-γ reversed the decline in IL-6 levels at 72 h, and in fact, the levels were threefold higher than those in cultures receiving pep M5 alone. In contrast to its effects on IL-1 production, exogenous IFN-γ by itself had no effect on IL-6 production by PBMC.

The pattern of TNF-α release by PBMC was very similar to that observed for IL-6 and followed the same kinetics, with high levels at 24 and 48 h and a sharp decline at 72 h. In the presence of added exogenous IFN-γ, the increased levels of TNF-α induced by pep M5 were sustained longer. At 72 h, pep M5-stimulated PBMC cultures contained 300 pg of TNF-α per ml, whereas cultures that received both pep M5 and IFN-γ had 1,000 pg/ml. Exogenous IFN-γ by itself had little or no effect on the production of TNF-α by PBMC.

We also examined the pattern of TNF-β production in response to pep M5 and found it to be different from the patterns of the other cytokines, with virtually undetectable levels at 24 h (Fig. 4). At 48 and 72 h, pep M5 alone induced the production of low levels of TNF-β, but IFN-γ alone had

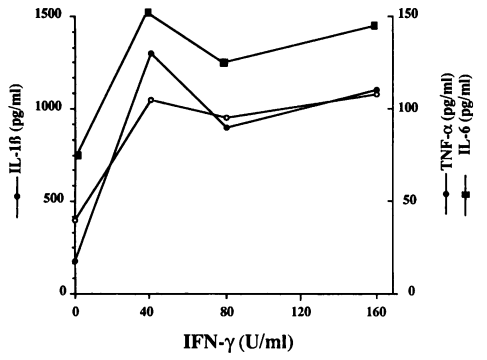

FIG. 2. Augmentation of cytokine release by pep M5-stimulated APC in the presence of various doses of IFN-γ. APC were seeded at 10^6 cells per ml in 24-well plates and cultured in RPMI complete medium for 48 h, and then the culture media were collected and centrifuged for 8 min at 400 × *g* to remove contaminating cells. Cultures were incubated with 1 μg of pep M5 per ml in the presence or absence of the indicated doses of IFN-γ. The culture supernatants were aliquoted and stored at −20°C until further analysis for cytokine activity as described in Materials and Methods. In the absence of pep M5, basal levels of IL-1β and TNF-α production were 230 and 39 pg/ml, respectively.

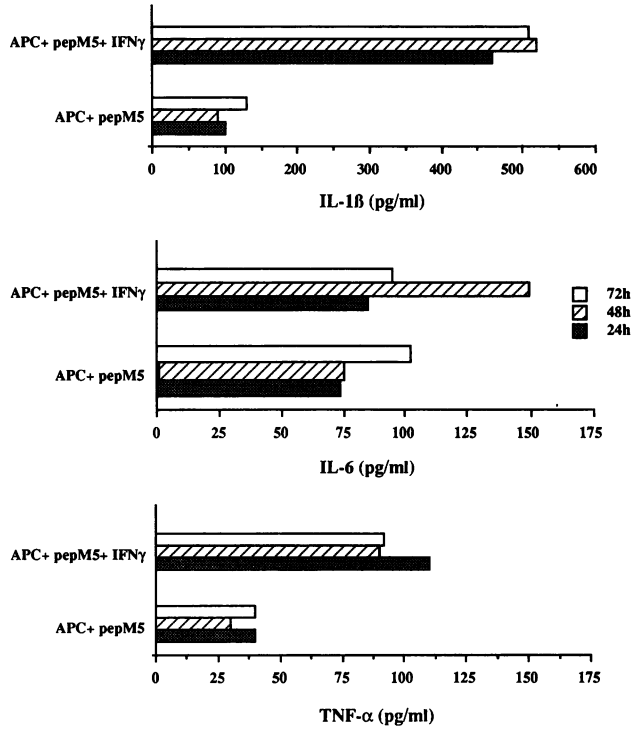

FIG. 3. Similarity in the pattern of cytokine release by APC from different individuals induced by pep M5 in the presence and absence of IFN-γ. Experimental conditions were identical to those described in the legend to Fig. 1 except that IL-1β was assayed by an ELISA.

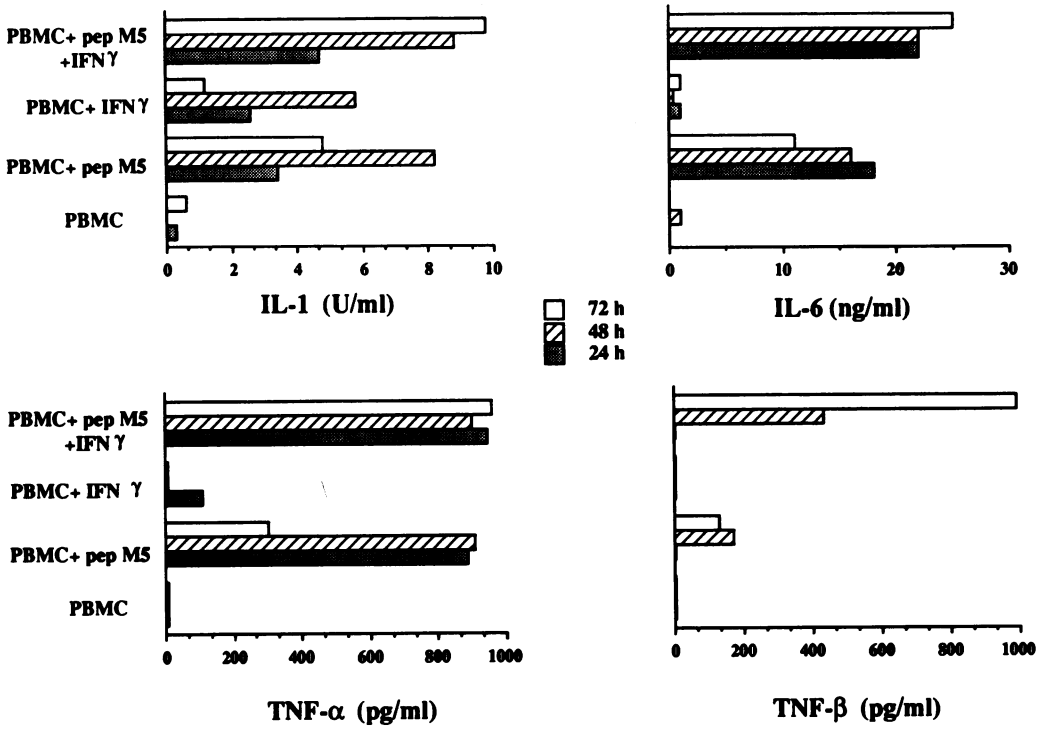

FIG. 4. Induction of cytokine release by pep M5-stimulated PBMC in the presence and absence of exogenous IFN-γ. PBMC were seeded at 10^6 cells per ml in 24-well plates and cultured in RPMI complete medium for 24, 48, or 72 h, and then culture supernatants were collected, aliquoted, and stored at −20°C until analysis for cytokine activity as described in Materials and Methods. As indicated, some cultures were stimulated with 1 μg of pep M5 per ml in the presence or absence of 100 U of IFN-γ per ml.

no effect on this cytokine. However, exogenously added IFN-γ acted synergistically with pep M5 in inducing TNF-β production by PBMC, which was not measurable at 24 h but increased to 400 pg/ml at 48 h and reached 1,000 pg/ml at 72 h.

Production of IFN-γ by pep M5-stimulated PBMC. Because pep M5 induced the production of high levels of cytokines in PBMC but not in APC cultures and because addition of IFN-γ to APC cultures augmented the release of inflammatory cytokines, we tested whether pep M5 might be inducing the production of IFN-γ by T cells. PBMC were incubated with pep M5, and at various time intervals, IFN-γ levels were measured. As shown in Fig. 5, pep M5 induced the production of IFN-γ, which at 72 h was 80-fold higher than in the absence of pep M5 stimulation.

Cytokine production by pep M5 and SEB. As mentioned above, pep M5 is a less potent superantigen than most bacterial toxins. It was of interest, therefore, to compare the quantitative and temporal aspects of cytokine production by pep M5- and SEB-stimulated PBMC. SEB-induced IL-1 levels were two- to fourfold higher than levels in pep M5-stimulated PBMC (Fig. 6). At 24 h, IL-6 levels reached 24 ng/ml in SEB-stimulated PBMC cultures and remained constant thereafter. The levels of IL-6 in SEB-stimulated PBMC were 1.5- to 2.5-fold higher than in pep M5-stimulated cultures.

The level of TNF-α produced in response to pep M5 was comparable to that of SEB at 24 and 48 h, although it declined considerably by 72 h, reaching 300 pg/ml (Fig. 6). However, in the presence of high levels of IFN-γ, pep M5-induced TNF levels remained elevated at 72 h (Fig. 4). Thus, the pattern of cytokine production by SEB-stimulated PBMC at 72 h resembled that obtained in cultures of pep M5-plus IFN-γ-stimulated PBMC. This suggested that the pattern observed with SEB may simply reflect the ability of this cytokine to induce higher levels of IFN-γ. Indeed, when we

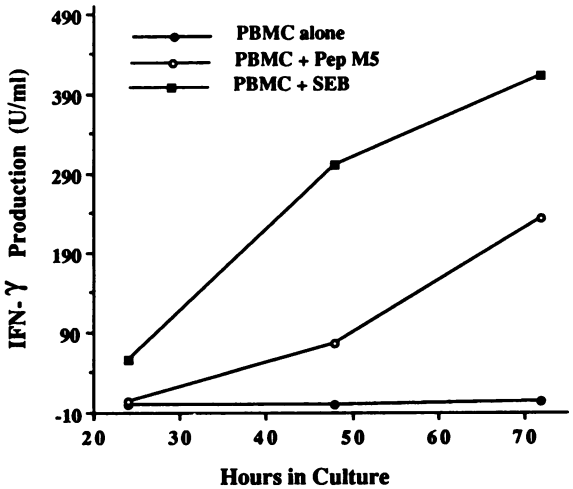

FIG. 5. Production of IFN-γ by PBMC stimulated with pep M5 or SEB. PBMC were seeded at 10^6 cells per ml in 24-well plates and cultured in RPMI complete medium alone, with 1 μg of pep M5 per ml, or with 1 μg of SEB per ml. At 24, 48, or 72 h, the culture supernatants were collected, aliquoted, and stored at −20°C until analysis for IFN-γ activity by a standard assay kit as described in Materials and Methods.

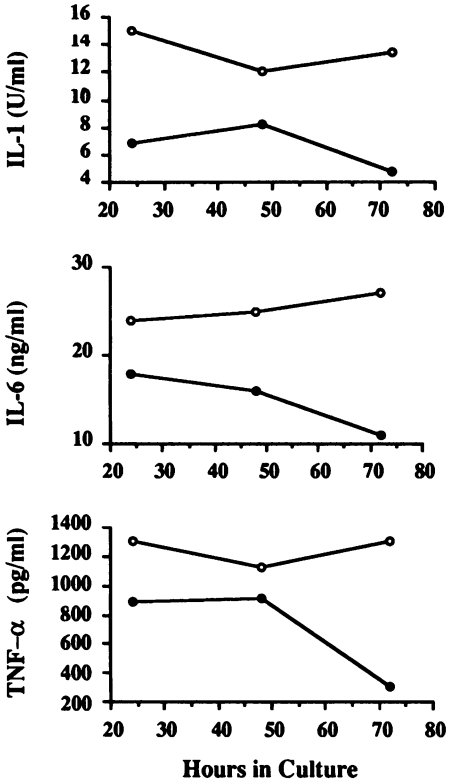

FIG. 6. Comparison of cytokine levels in cultures of PBMC stimulated with pep M5 (●) or SEB (○). PBMC were seeded at 10^6 cells per ml in 24-well plates and cultured in RPMI complete medium alone, with 1 μg of pep M5 per ml, or with 1 μg of SEB per ml. At 24, 48, or 72 h, the culture supernatants were collected, aliquoted and stored at −20°C until analysis for cytokine activity as described in Materials and Methods.

measured IFN-γ production in SEB-stimulated PBMC, we found it to be 14-, 4-, and 2-fold higher than in pep M5-stimulated cultures at 24, 48, and 72 h, respectively (Fig. 5).

DISCUSSION

Superantigens interact with specific elements within the Vβ portion of the T-cell receptor for antigens (6, 22, 25, 31, 34). Consequently, all T cells bearing those particular elements are induced to proliferate in response to superantigens. The responding T cells have a wide array of antigenic specificities directed against host tissues. This has led several groups to hypothesize that exposure to superantigens in vivo can potentially lead to autoimmunity. Still, the role of superantigens in the pathogenesis of postinfectious autoimmune diseases is poorly understood. It is well documented, however, that superantigens are powerful inducers of cytokines, which orchestrate intercellular interactions and regulate immune responses (1a, 12, 13, 18, 24, 26, 38).

To better understand the role of M protein in the pathogenesis of streptococcal autoimmune diseases, we investigated its ability to induce the production of a number of inflammatory cytokines. As shown in this study, the ability of pep M5 to induce the production of TNF-α, IL-1, and IL-6 was dependent on the presence of either T cells or T-cell-derived factors. These data are similar to previously reported results showing that the ability of staphylococcal superantigen SEA to induce production of IL-1 (18, 41a) or TNF-α (14) by human monocytes required the participation of T cells. However, in contrast to these reports, we found that the requirement for T cells can be met by exogenous IFN-γ. This cytokine regulates the production of other cytokines, and as suggested by Pischedda et al. (38a) and Grossman et al. (20), IFN-γ can up regulate the expression of major histocompatibility complex class II molecules, thereby enhancing the responsiveness of monocytes and T cells to superantigenic stimulation.

Cooperation between cytokines is required for mounting physiologic immune and inflammatory responses. The data presented in this study clearly show that IFN-γ was required for pep M5-induced production of IL-1, IL-6, and TNF-α by a T-cell-depleted APC population. The ability of IFN-γ to augment the production of these cytokines has been shown in a number of systems (5, 20, 40, 51). Sanceau et al. (40) showed that TNF-α-induced IL-6 secretion by monocytic cells required IFN-γ. A similar effect on IL-1 and TNF-α production was reported by Burchett et al. (5), who demonstrated that IFN-γ-primed monocytes produced significantly higher levels of these cytokines in response to lipopolysaccharide. Likewise, Turner et al. (51) demonstrated that TNF and IFN-γ synergistically induce the transcription of IL-1α and IL-1β genes. The augmentation of cytokine production by IFN-γ in our system cannot be attributed to contaminating lipopolysaccharide, because in the presence of IFN-γ alone, T-cell-depleted mononuclear cells secreted no or insignificant quantities of cytokines. The increase in IL-1 production in PBMC cultures treated with IFN-γ alone can be attributed to the fact that IFN-γ directly enhances IL-1 production (11).

Despite variations in the overall levels of cytokine production from one individual to another, the ability of exogenous IFN-γ to potentiate cytokine production was consistently observed. The kinetics and temporal relationship of cytokine release were also similar from one individual to another. The decline following peak release of inflammatory cytokines seen here has been observed both in vivo and in vitro in other systems and could be attributed to either a decrease in synthesis or an increase in consumption due to up regulation of cytokine receptors followed by intracellular uptake. The data presented here do not distinguish between these possibilities. Since our studies were performed with cells from healthy individuals, it would be important to determine whether the same patterns are observed with cells from patients suffering from streptococcal infections or their long-term sequelae. The ability to release certain levels of cytokines or to express relevant receptors may be an important factor in the susceptibility of certain individuals to postinfection immunological disorders.

In addition to the synergistic effects of IFN-γ on the production of IL-1, IL-6, and TNF-α by pep M5, these cytokines have been shown to regulate each others' production. For example, TNF-α augments the production of IL-1 and IL-6, but IL-6 can suppress the production of TNF-α and IL-1 (1), and IL-1 has been shown to down regulate TNF-α receptors (17, 23). This may in part explain our findings that in cultures of T-cell-depleted mononuclear cells, levels of TNF-α declined as levels of IL-1 and IL-6 increased.

It is clear from this study that the participation of T cells is essential to the ability of pep M5 to induce cytokine production. In the presence of T cells, pep M5 induced measurable levels of IL-1, IL-6, and TNF-α and low levels of TNF-β. Although in most cases the addition of exogenous

IFN-γ at the onset of culture did not affect the overall levels of cytokine production, it did alter the kinetics of cytokine release. In all cases, IFN-γ prevented the late decline in cytokine levels and sustained the levels for a prolonged period. In this regard, our data are similar those of Haq (21), who found that priming aged monocytes with IFN-γ prevented the loss in IL-1 secretory potential. Similarly, Mackensen et al. (33) reported that in vivo administration of IFN-γ to cancer patients receiving intravenous injections of endotoxin not only prevented cytokine down regulation but also caused the production of TNF and IL-6.

Grossman et al. (20) suggested that the ability of IFN-γ to augment cytokine production in response to superantigenic stimulation may be attributed in part to its ability to up regulate the expression of major histocompatibility complex class II molecules, thereby enhancing the responsiveness of B cells and monocytes to superantigen. Although the ability of pep M5 to induce the production of inflammatory cytokines may be related to its ability to induce the production of IFN-γ, it is clear that this superantigen is providing signals to both T cells and APC, which then act synergistically with IFN-γ, and possibly with other T-cell-derived factors as well, to augment cytokine responses. Of particular interest was the marked augmentation of TNF-β production that occurred only in the presence of pep M5 and exogenous IFN-γ. Recently, Anderson et al. (1a) showed that TNF-β is released in response to SPEA and SEA but not in response to lipopolysaccharide stimulation. This cytokine may play an important role in gram-positive shock and may serve as a useful marker to differentiate between gram-negative and gram-positive toxic shocks. The kinetics of IL-1, IL-6, TNF-α, and TNF-β release may vary depending on the amount and/or timing of IFN-γ release, because the patterns in the presence of exogenously added and endogenously produced IFN-γ are different. The abilities of IFN-γ to augment the production of cytokines (8) and nitric oxide (30) and to increase the expression of cell adhesion molecules (32) is bound to exacerbate the immune response. Thus, the secretion of high levels of IFN-γ in an individual who is undergoing a viral infection at the time of exposure to the streptococcal superantigen may show a completely different cytokine profile than that in a patient with a low basal level of IFN-γ. It is possible that the response to infections is determined by the quality, quantity, and timing of cytokine production, which could be influenced by genetic and/or environmental factors. Thus, the cytokine profile may be a predisposing factor for the development of nonsuppurative sequelae.

Abnormalities in cytokine production have been reported for a number of autoimmune diseases (9, 10, 19) including rheumatic heart disease (36), but it is not clear whether these abnormalities are a cause or consequence of these disorders. Inasmuch as superantigens are potent producers of cytokines (13, 18, 20, 24, 26, 35, 38), their possible role in autoimmune disorders may be related to the type and quantity of the cytokine produced. It is interesting that the two superantigens that have long been associated with autoimmune diseases, the M protein and MAM, are less-potent T-cell mitogens than the pyrogenic toxins (16, 31). Overstimulation by potent pyrogenic toxins can either kill or inactivate cells. This has been demonstrated by recent in vivo findings of superantigen-induced programmed cell death (28) or anergy (37, 39). It is conceivable that cells exposed to less-potent superantigens would stand a better chance of surviving the immune insult, thereby maintaining their abilities to respond to either foreign or self antigens. In this study, we showed that although the levels of TNF-α induced by pep M5 were comparable to those induced by SEB, SEB was a more potent producer of IL-1 and IL-6. In fact, the patterns of IL-1, IL-6, and TNF-α release were very similar to those observed in cultures stimulated with pep M5 and exogenous IFN-γ. This probably reflects the fact that SEB was a much more potent inducer of IFN-γ production.

In summary, we have shown that the streptococcal superantigen pep M5 is a potent stimulator of cytokine production and that this activity requires the participation of T cells. Inasmuch as this superantigen has been implicated in the pathogenesis of rheumatic fever, rheumatic heart disease, and glomerulonephritis, understanding the temporal relationship of cytokine induced by this superantigen may clarify the role of this superantigen in these autoimmune diseases.

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