Inflammation, Vol. 21, No. 5, 1997

CYTOKINE RESPONSES OF HUMAN BLOOD MONOCYTES STIMULATED WITH Igs

JAMI L. FOREBACK, DANIEL G. REMICK, ELAHE CROCKETT-TORABI, and PETER A. WARD

Department of Pathology, University of Michigan Medical School Ann Arbor, Michigan 48109

Abstract—Using an in vitro system for stimulating human peripheral blood mononuclear cells (PBMC) with immobilized Ig, patterns of cytokine production as a function of different Ig classes and subclasses were elucidated. Wells were coated with IgA, IgG1, IgG2, IgG3 or IgG4. Equivalent protein content on surfaces of wells was demonstrated by a human kappa chain ELISA. Isolated human PBMC were added to Ig-coated wells and incubated for 24 hrs before supernatants were assayed for cytokines. The IgG subclasses showed differences in cytokine production stimulated from PBMC, with the relative stimulation for TNFα being IgG2 $\geq$ IgG3 $\geq$ IgG1 > IgG4 and for IL-6 production, IgG2 $\geq$ IgG3 > IgG1 = IgG4. In contrast, the relative stimulation for IL-8 was IgG1 = IgG2 = IgG3 = IgG4. IgA caused less production of TNFα when compared to IgG2, but similar levels of IL-8. Such differences may have important implications in the pathogenesis of immune complex mediated diseases.

INTRODUCTION

Since the discovery of the IgG subclasses, much has been learned about the structure and biochemistry of these molcules (1–3), whereas much less is known about their biological function. The subclasses are similar in molecular weight but differ in their amino acid sequences and functional activities. For example, IgG1 and IgG3 are superior to IgG2 and IgG4 for activation of complement. IgG1 and IgG3 are produced predominately in response to protein antigens, while IgG2 is produced chiefly in response to carbohydrate antigens (4). Some clinical correlations have been found, such as susceptibility to encapsulated bacteria (such as *H. influenza* and *Streptococcus pneumoniae*) in individuals with low serum levels of IgG2 (4–6), the association of IgG4 production with the synthesis of IgE and allergic disorders (4, 7, 8), and correlations between disease onset and deposition of subclasses of Igs in glomerulonephritis (4, 6), inflammatory bowel diseases (9, 10) and systemic lupus erythematosus (11). The

0360-3997/97/1000-0501$12.50/0 ©1997 Plenum Publishing Corporation

pathogenic mechanisms underlying these associations are still largely unknown. Since cytokines mediate many inflammatory responses, their production may be a key step for induction of disease following Ig deposition in tissues. For instance, patients with IgA-associated nephropathy, alcoholic liver cirrhosis or acquired immune deficiency syndrome show increased serum levels of IL-1, TNFα and IL-6 (12). In this report, we have assessed the relative in vitro ability of IgG subclasses and IgA to induce cytokine production from human peripheral blood mononuclear cells (PBMC). The data show some strikingly different patterns of cytokine responses as a function of the class or subclass of Ig employed and the cytokine under analysis.

MATERIALS AND METHODS

Cell Isolation. PBMC were isolated from the venous blood of healthy volunteers by separatoin over Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) followed by two washes with 0.9% sodium chloride (Abbott Laboratories, North Chicago, Illinois). Cells were resuspended in Dulbecco's Modified Eagle Medium (Gibco BRL, Gaithersburg, Maryland) containing 10% fetal bovine serum, 1% L-glutamine, 1% antibiotic/antimycotic and 1% non-essential amino acids (DMEM 10%). Cells were used at a concentration of 2×10^6 per ml unless otherwise noted. PBMC were used rather than purified monocytes because of the difficulty of obtaining unstimulated pure cells and the physiological relevance of a mixed cell population.

Reagents. Human myeloma IgG subclasses (all kappa light chain) purified from plasma were purchased from Sigma (St. Louis, Missouri). Lipopolysaccharide from *Escherichia Coli* serotype 0111:B4 and low endotoxin bovine serum albumin (BSA) were also purchased from Sigma. Purified human serum IgA was purchased from Organon Teknika-Cappel (Durham, North Carolina).

Coating of Plates for Cell Stimulation or Kappa Chain ELISA. 96-well Immulon plates (Dynatech Inc., Chantilly, Virginia) were coated overnight at 4°C with indicated amounts of human IgG subclasses or BSA. All antibodies were spun for five min at 14,000 rpm before use to remove any nonspecific aggregates. Dilutions were made in sterile phosphate buffered saline (PBS) and 50 μl plated per well. IN experiments comparing subclasses, equivalent binding was determined using an ELISA to detect human kappa light chain. Thus, plates were coated in duplicate, one for cell stimulation and one for the human kappa chain ELISA to compare immunoglobulin subclass binding.

Human Kappa Light Chain ELISA. Antibody-coated plates were analyzed with an ELISA to detect the human kappa light chain. Plates were washed five times in 250 μl of PBS before a combination blocking/primary antibody step was performed. In that step, biotinylated anti-human kappa chain antibody (Sigma) was diluted $1:2 \times 10^4$ in a 5% solution of nonfat dry milk and 200 μl added per well. The plate was rocked for 45 min at room temperature before washing as above. 100 μl per well of a 1:3000 dilution of strep-avidin conjugated to horseradish peroxidase (Zymed Laboratories Inc., S. San Francisco, California) was then added and incubated for 30 min. Plates were washed five times and 200 μl per well of an o-phenylenediamine dihydrochloride (OPD) substrate (Sigma) was added. The reaction was stopped after 3–5 min by adding 50 μl per well of 3M H_2SO_4. Plates were read at 490 nm on an automated plate reader (Biotek Instruments, Winooski, Vermont). Mean absorbances of triplicate wells were used to compare binding.

Cell Stimulation. IgG, IgA, or BSA-coated plates were incubated with sterile PBS, in parallel

with plates undergoing the kappa chain ELISA, before addition of cells. Briefly, plates were washed with 250 μl/well of PBS five times and then rocked at room temperature with 200 μl/well of PBS for 45 min. Plates were then washed and rocked at room temperature with 100 μl/well of PBS for 30 min. Plates were washed once again before 200 μl/well of cells at 2×10^6/ml were added. Incubation was at 37°C in a humidified, 5% CO_2 incubator for 24 hr unless otherwise indicated. After incubation, supernatants were harvested in 1.5 ml tubes, spun for 10 min at 14,000 rpm to pellet any contaminating cells and the supernatants placed in fresh tubes until cytokine analysis, typically the same day. Otherwise, supernatants were frozen at −20°C until analysis.

Proteinase K Digestion. A 20 μg/ml solution of proteinase K (Promega, Madison, Wisconsin) in Hank's Balanced Salt Solution (HBSS) was used to dilute IgG subclasses and IgA to 0.5 mg/ml and LPS to 0.1 mg/ml. Control samples were diluted in HBSS alone and all treatments were incubated overnight at 37°C. The following day samples were kept on ice and the extent of digestion was analyzed by SDS-PAGE followed by Coomassie staining. Plates were then coated with 100 μl (50 μg) of each digested or intact antibody, BSA or 200 μl (20 μg) of enzyme-treated or control LPS. Cell stimulation was then carried out as described.

Fc Receptor Blocking. After isolation of PBMC, cells were suspended to 10×10^6 cells per ml in DMEM 10%. Cells were aliquotted for pretreatment with sterile PBS, mouse Fab fragments (Accurate, Westbury, New York), the mouse monoclonal 32.2 $F(ab')_2$ against FcγRI or the mouse monoclonal IV.3 Fab against FcγRcII (Medarex, Annandale, New Jersey). All Fab fragments were centrifuged at 14,000 rpm for 5 min to remove aggregates, then used at a concentration of 100 μg/ml. Cells were pretreated for 30 min on ice, then diluted to 2×10^6 per ml in DMEM 10%, spun for 10 min at 1000 rpm to wash off unbound antibody and resuspended at 2×10^6 per ml. 200 μl/well of treated cells were then added to an antibody-coated plate, incubated for 4 hr, and supernatants harvested as described under "Cell Stimulation."

LPS Assessment. LPS contamination of the commercial human IgG subclasses and IgA was assessed using the Limulus Amebocyte Lysate Assay (BioWhittaker, Inc., Walkersville, Maryland). LPS levels were typically < 1 ng/ml, those above 1 ng/ml were tested side-by-side with equivalent amounts of LPS. TNFα levels resulting from LPS equivalent to the amount of Ig contamination were one-tenth that of the Ig stimulation.

Cytokine Quantitation. TNFα protein was assessed using a sandwich ELISA as described (13). The minimum level of detection was 1 ng/ml. IL-8 protein was quantitated using a modified sandwich ELISA (14) and IL-6 was quantitated using a B9 bioassay (15). Measurement of MCP-1 was performed by ELISA as previously described (16).

Statistics. Statistical analysis was performed using SigmaStat (Jandel Corp., Sausalito, California). Data satisfying the requirements for normality were analyzed using a one way analysis of variance (ANOVA) followed by the Student-Newman-Keuls method for pairwise comparisons. Data failing the normality test were analyzed using a Kruskal-Wallis one way ANOVA on ranks followed by the Student-Newman-Keuls method for pairwise comparisons. Statistical significance was recognized as $p < 0.05$.

RESULTS

Enzymatic Degradation of IgG Subclasses and Abrogation of TNFα Production. Preliminary data suggested that several IgG subclasses were capable of stimulating TNFα production in PBMC (Figure 1). In these experiments, the rank order of PBMC stimulation was: IgG2 > IgG3 ≥ IgG1 ≫ IgG4. To deter-

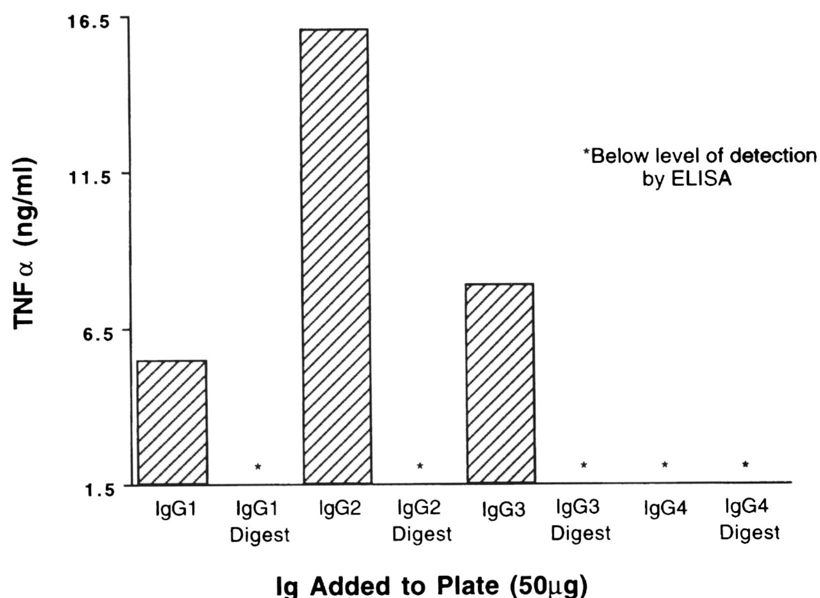

Fig. 1. Effect of proteinase K digestion on IgG subclass stimulation of TNFα. IgG subclasses were incubated overnight with proteinase K or buffer, then assessed for digestion by SDS-PAGE. 50 μg of digested or intact subclasses were then used to coat a 96-well plate for cell stimulation.

mine whether the ability of IgG subclasses to stimulate PBMC required intact Ig or might be due to LPS contamination, the subclasses were digested with the proteolytic enzyme proteinase K and then incubated with microtiter wells prior to addition of PBMC. This treatment caused extensive degradation of IgG proteins into low molecular weight fragments as determined by gel electrophoresis (data not shown). After digestion, the ability of each subclass to stimulate TNFα production was abolished (Figure 1), implying that contaminating LPS was not responsible for the observed findings. As a further control, purified LPS was treated with proteinase K. Figure 2 shows that, as would be predicted, LPS treated in this manner was still an effective stimulus for induction of TNFα production by PBMC. Experiments performed on IgA showed results similar to the data with IgG subclasses (data not shown).

From the data in Figure 1, IgG2 appeared to be the best stimulus for TNFα production. However, differences in activities of IgG subclasses might have been due to differences in amount of protein adhering to surfaces of microtiter wells. To directly compare the ability of IgG subclasses to stimulate cytokine production, 25 μg of each subclass were coated onto wells. Approximately equivalent binding of IgG subclasses to plastic surfaces was demonstrated using an anti-

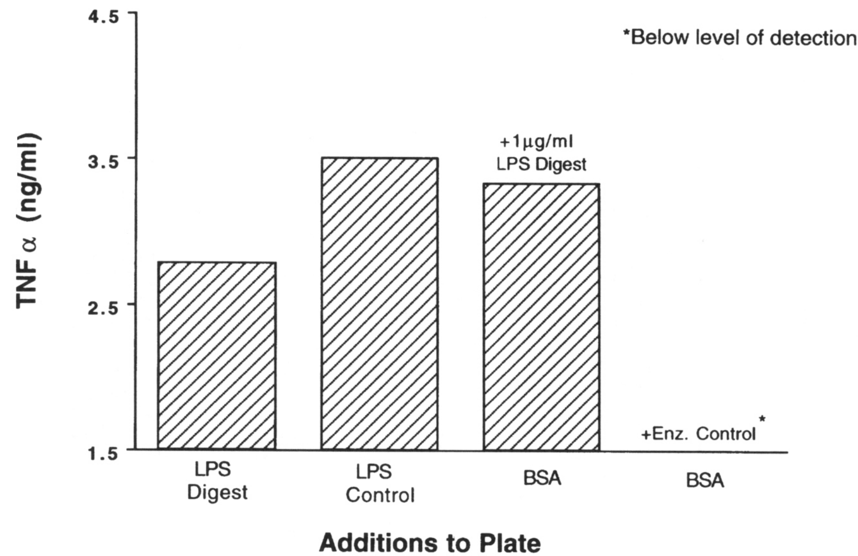

Fig. 2. Effect of proteinase K treatment on LPS stimulation of TNFα. LPS was incubated overnight with proteinase K or buffer. Enzyme or buffer-treated LPS, or BSA, was then used to coat a plate for cell stimulation. In addition, digested LPS or enzyme solution was added to BSA-coated wells upon addition of cells to the wells. Bar 1 is TNFα stimulation by proteinase K-treated LPS coating the well. Bar 2 is TNFα stimulation by buffer-treated LPS coating the well. Bar 3 is a BSA-coated well stimulated by addition of proteinase K-treated LPS with the cells. Bar 4 is a BSA-coated well treated with only the enzyme solution added to the cells.

human kappa light chain ELISA (Figure 3). Thus, any differences in cytokine production induced by various IgG subclasses could not be attributed to differences in protein adherence to plastic surfaces.

Patterns of TNFα, IL-6, and IL-8 Production in Response to IgG Subclasses. IgG1, IgG2, IgG3, and IgG4 were evaluated for their ability to stimulate expression of TNFα, IL-6, and IL-8. The results are shown in Figure 4. For TNFα, IgG2 was more stimulatory than IgG4 (16.4 ± 4.0 vs 2.7 ± 0.9, $p < 0.05$), while in these experiments TNFα production in response to IgG1 and IgG3 could not be statistically discriminated from IgG2-induced production of TNFα. This result agrees with that shown in Figure 1. Under the same experimental conditions, in which supernatant fluids were analyzed for IL-6, IgG2, and IgG3 were more stimulatory than IgG1 or IgG4 (difference of ranks = 47.5 for IgG2 vs IgG1, 42.0 for IgG2 vs IgG4, 34.5 for IgG3 vs IgG1, 29.0 for IgG3 vs IgG4; $p < 0.05$). In striking contrast, the response of IL-8 was much more robust, with no distinguishable differences for any of the IgG subclasses tested (Figure 4C). As will be shown in Figure 6, the pattern of cytokine responses to IgG subclasses

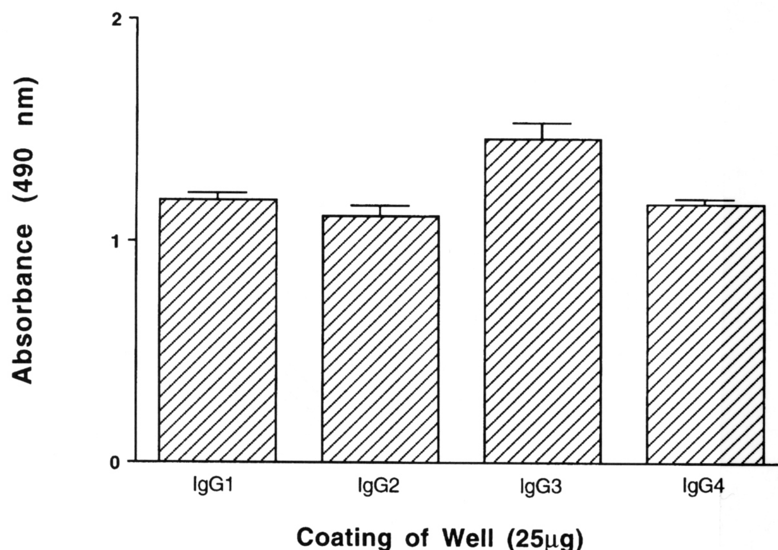

Fig. 3. Comparison of IgG subclass binding using an ELISA to detect human kappa chain. A 96-well plate was coated with the IgG subclasses in a manner identical to plates used for cell stimulation (Figure 4). The coated plate was then subjected to an ELISA to detect human kappa light chain. Raw absorbances were used to compare binding, as the subclasses used in this study were all kappa chain. Bars represent the average absorbance of triplicate wells with the *SEM*.

was consistent across a range of doses of IgG subclasses. The amount of IL-8 produced in response to IgG subclasses was typically 5–20 fold higher than the levels of TNFα or IL-6 produced. In the same experiments, MCP-1 levels were assessed but were too low for detection (data not shown).

To assess the time course for cytokine responses of stimulated PBMC, cell culture supernatant fluids were collected at 4, 8, 12 and 24 hr (Figure 5). In frame A the TNFα response reached a plateau between 4 and 8 hr except in the response to IgG1, which peaked at 24 hr. As shown in Figure 5, the rank order of TNFα responses was consistent with data in Figure 4: IgG2 > IgG3 > IgG1 ≥ IgG4. In Figure 5, frame B, IL-8 was produced at consistently high levels with no discriminant differences in responses to various IgG subclasses. Again, no statistically significant differences between IgG subclasses were found for IL-8 production. These results indicate that the IgG subclasses show consistent differences in their ability to stimulate TNFα, whereas the IL-8 response is similar for all subclasses of IgG.

Cytokine Responses as a Function of Amount of IgG Subclass Employed. To evaluate the effect of increasing amounts of IgG subclasses to stimulate cytokine production, subclasses were compared at 0.5, 5, and 25 μg. In Fig-

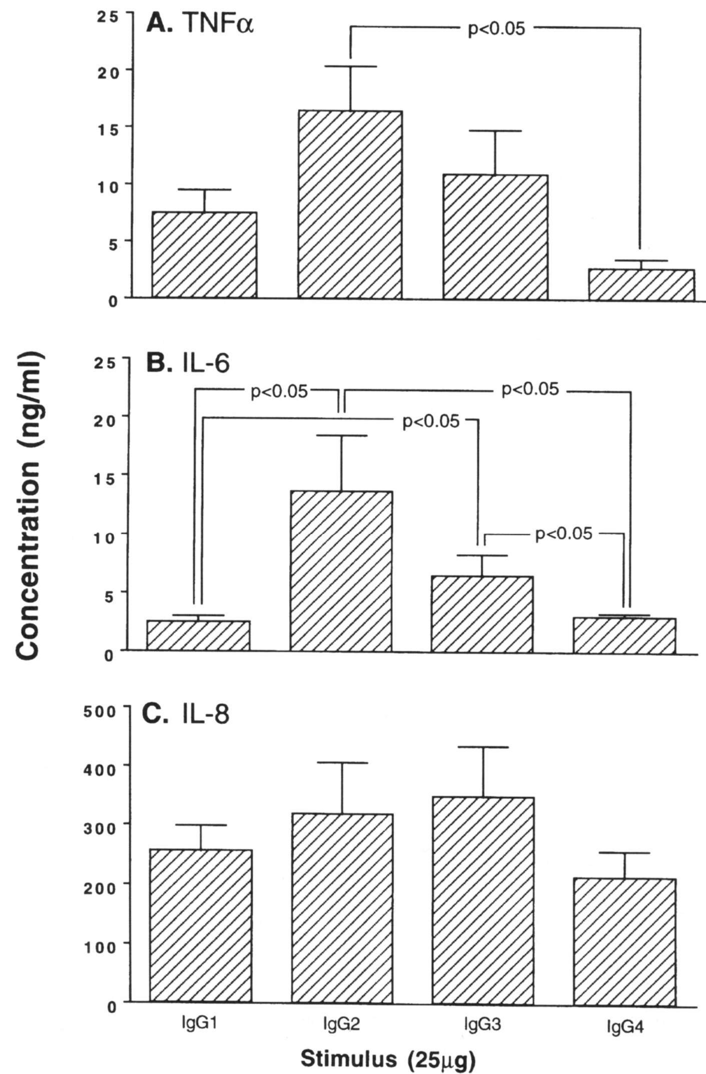

Fig. 4. Cytokines produced by PBMC in response to IgG subclasses. Plates were coated overnight with 25 μg of IgG subclasses, washed, and cells in medium were added. After 24 hr at 37°C, supernatants were harvested for assessment of cytokine content. Bars represent the mean of four experiments with the SEM. Significance was recognized as $p < 0.05$.

ure 6, TNFα and IL-6 levels were low to undetectable with 0.5 μg. At 5 μg, the rank order of IgG subclass ability to stimulate TNFα and IL-6 production was similar to the pattern found with 25 μg, also shown in Figure 4. For IL-8 pro-

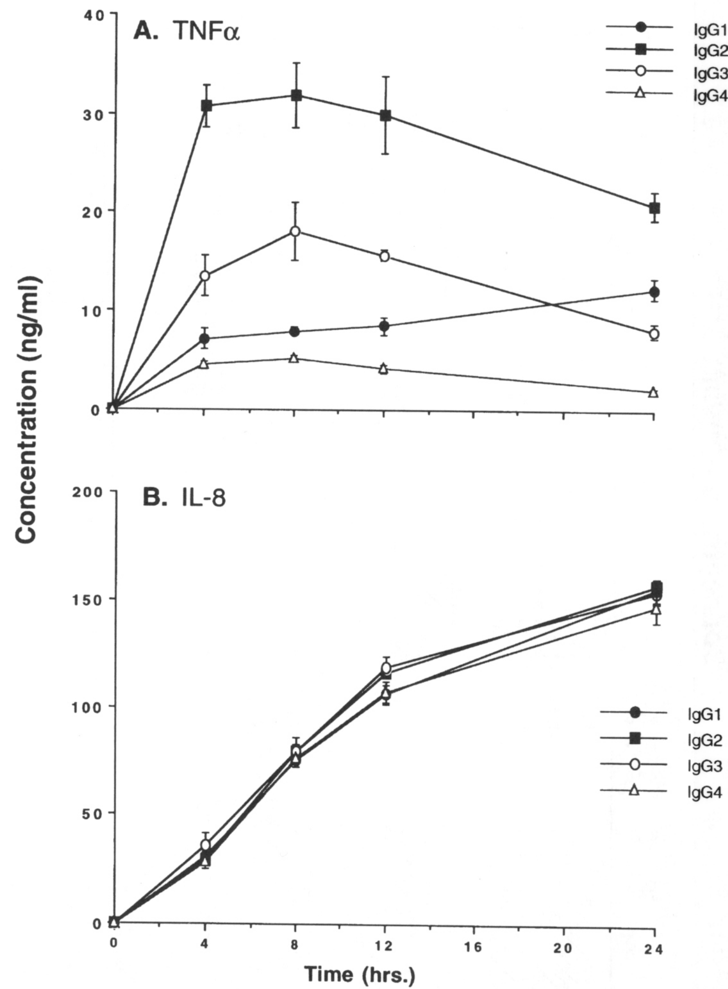

Fig. 5. Time course of TNFα and IL-8 production by human PBMC in response to IgG subclasses. Plates were coated overnight with 25 μg of IgG subclasses, washed and cells in medium were added. At various time points, supernatants were havested and frozen for assessment of cytokine content. Each point represents the average of triplicate wells with the *SEM*.

duction, any differences between the subclasses at the 0.5 μg dose were clearly gone at the 5 μg and 25 μg doses, confirming the findings in Figures 4 and 5. In other experiments, IgG4 when coated at 50, 100, and 200 μg never induced TNFα production that exceeded 5.5 ng/ml, demonstrating the relative inability of IgG4 to stimulate TNFα. Thus, the differential abilities of the IgG subclasses

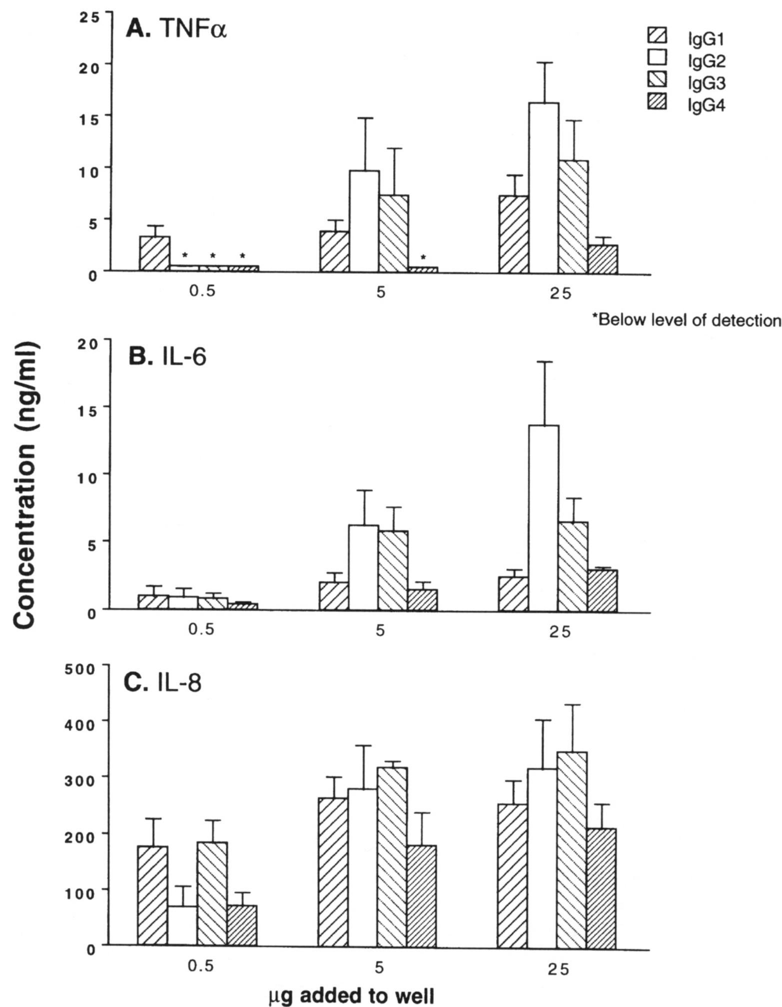

Fig. 6. Cytokines produced by PBMC in response to 0.5, 5 or 25 μg of IgG1, IgG2, IgG3 or IgG4. Plates were coated overnight, washed and cells in medium added. After 24 hr at 37°C, supernatants were harvested for assessment of cytokine content. Bars represent the mean of four experiments with the *SEM*.

to stimulate TNFα and IL-6 production hold even at the higher doses of IgG subclasses employed.

TNFα, IL-6, and IL-8 Responses to IgG2 and IgA. To assess the ability of human serum IgA to stimulate cytokine production, IgA was compared to IgG2 under conditions identical to those shown in Figures 4 and 5. In Figure 7, the

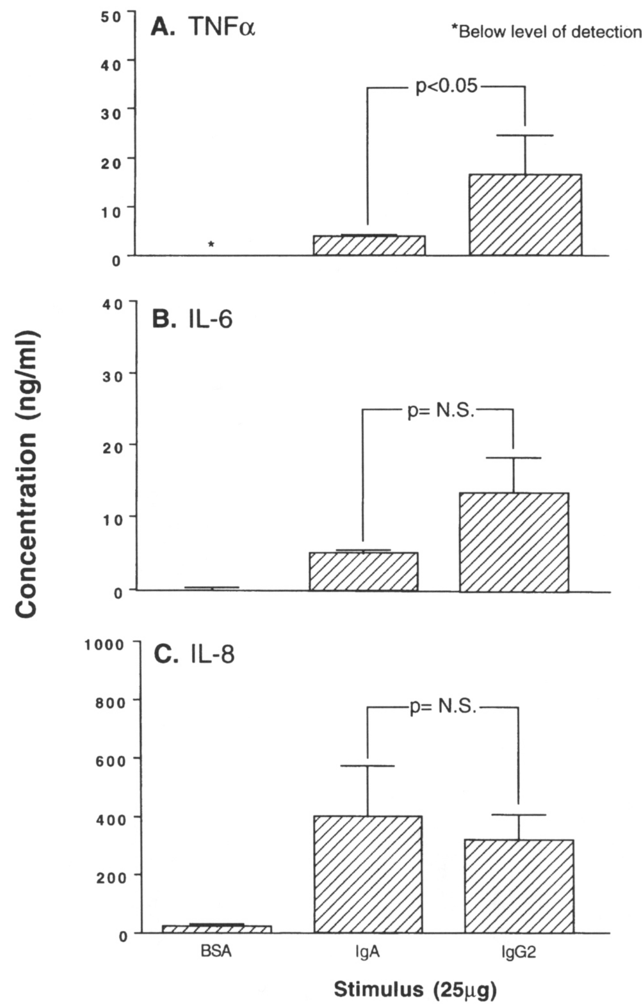

Fig. 7. Cytokines produced by PBMC in response to BSA, IgA or IgG2. Plates were coated overnight with 25 μg of BSA, IgA or IgG2, washed and cells in medium added. After 24 hr at 37°C, supernatants were harvested for assessment of cytokine content. Bars represent the mean of four experiments with the SEM. p = N.S. indicates values are not statistically different.

cytokines produced in response to IgA or IgG2 were compared to the responses to BSA-coated wells. Frame A showed that IgG2 was a better stimulus for TNFα production when compared to IgA. In this case, a statistically significant difference was achieved between IgG2 and IgA (16.4 ± 4.0 vs 3.8 ± 0.4, respectively, $p < 0.05$). With respect to IL-6 and IL-8 production, there were no statistically

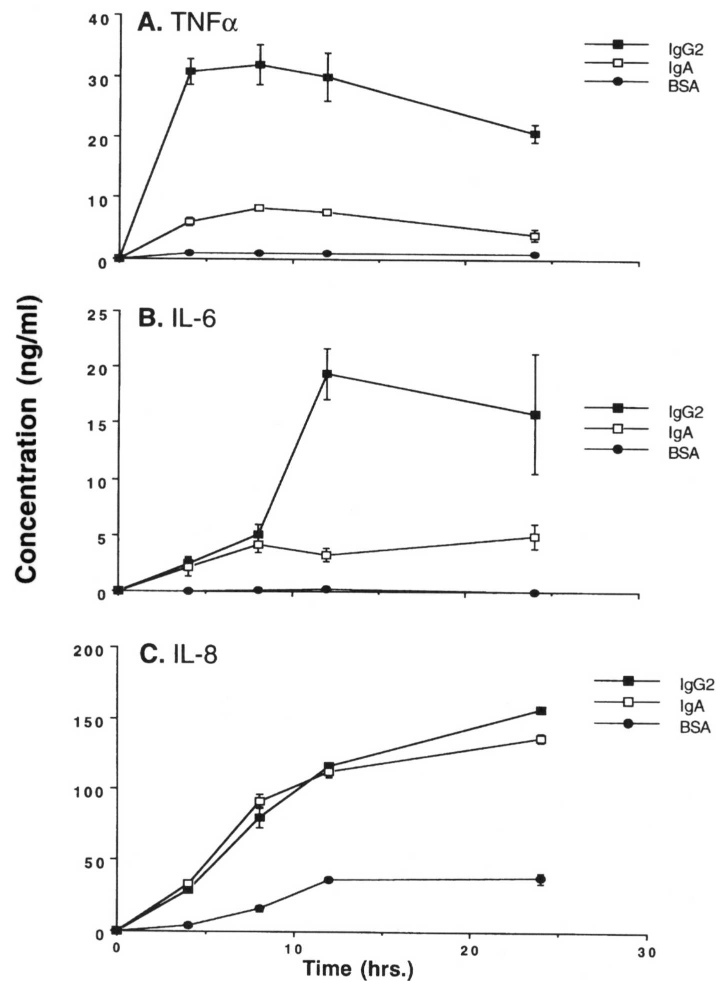

Fig. 8. Time course of cytokine production by human PBMC in response to 25 μg of IgG2, IgA or BSA. Plates were coated overnight, washed and cells in medium were added. At various time points, supernatants were harvested and frozen for assessment of cytokine content. Each point represents the average of triplicate wells with the *SEM*.

significant differences between cytokine responses of PBMC to IgA and IgG2. When compared over a time course (Figure 8), these trends for TNFα and IL-8 production persisted, with IgG2 consistently being a better stimulus than IgA for TNFα production. IL-6 production showed no impressive differences with respect to IgG2 and IgA for the first 8 hr, while at 12 and 24 hrs IgG2 was

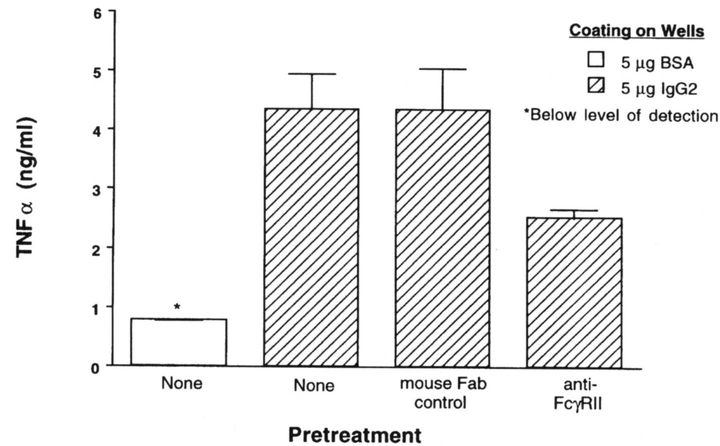

Fig. 9. TNFα production following blockade of PBMC FcγRII. Cells were pretreated for 30 min with medium (Bars 1 and 2), 100 μg/ml of a control mouse Fab fragment (Bar 3), or 100 μg/ml of the anti-FcγRII IV.3 Fab (Bar 4). After pretreatment, cells were washed and added to wells coated with 5 μg of BSA or IgG2. Following a 4 hr incubation, supernatants were harvested and assessed for TNFα. Bars represent the average of triplicate wells with the SEM. Results represent one of four experiments.

a superior stimulus for IL-6 production when compared to IgA. In the case of IL-8, the response to IgA and IgG2 was the same (Figure 8C).

Role of FcγRII in TNFα Response of PBMC to IgG2. To determine if PBMC stimulation was occurring through an Fc receptor, Fc receptor blocking studies were performed using immobilized IgG2 as the stimulus and employing antibodies to human FcγRI and FcγRII. In dose-response experiments with a 4 hr cell stimulation, blocking, when found, was maximal at an antibody concentration of 100 μg/ml (data not shown). Anti-FcγRI $F(ab')_2$, which does not block activation of FcγRI (17), did not have an inhibitory effect on TNFα or IL-8 production (data not shown). Anti-FcγRII Fab effects on TNFα responses of PBMC to IgG2 were studied (Figure 9). Anti-FcγRII reduced the TNFα response of PBMC by approximately 40%. These studies demonstrate that TNFα production by PBMC is stimulated by interaction with solid phase immunoglobulin via FcγRII interaction with PBMC.

DISCUSSION

Little is known about the ability of subclasses of immunoglobulins to stimulate PBMC for cytokine/chemokine production and the relevance of this phe-

Table 1. Summary of Cytokine Results

Cytokine produced[a]	Relative stimulation[b]
TNFα	IgG2 ≥ IgG3 ≥ IgG1 > IgG4
IL-6	IgG2 ≥ IgG3 > IgG1 = IgG4
IL-8	IgG1 = IgG2 = IgG3 = IgG4

[a]The cytokine assayed following a 24 hr stimulation of PBMC by immobilized IgG subclasses.
[b]The relative ability of the IgG subclasses (25 μg) to stimulate cytokine production from PBMC.

nomenon to induction of human disease by Ig deposition. The ability of IgG1, IgG2, IgG3, IgG4 and IgA to activate PBMC appears to depend upon which cytokine (or chemokine) is examined (Table 1), implying that cytokine production in response to Ig subclasses must be via different signal transduction pathways. For TNFα production, dramatic differences in stimulation were apparent, particularly between IgG2 and IgG4. A similar pattern of stimulation followed in the case of IL-6, with IgG2 and IgG3 proving to be superior to IgG1 and IgG4. For IL-8 production, however, the pattern was very different. The IgG subclasses and IgA, which was a relatively weak stimulus for TNFα production as compared to IgG2, stimulated IL-8 production approximately equally. This result demonstrates that IgG4 is capable of functioning as a stimulus and that its inability to stimulate production of TNFα and IL-6 is not the result of an inactive IgG subclass. Since the IgG subclasses employed were obtained as myeloma proteins, the possibility exists that the properties displayed are not generalizable to IgG subclasses. However, multiple lots of IgG2 (each from a different donor) were found to be consistently superior for stimulation of TNFα production. IgG subclasses purifed from normal human serum vary in their light chain composition, making comparisons problematical.

The reasons for the disparate responses in TNFα, IL-6, and IL-8 production are unclear. The differing responses may be secondary to differences in Fc receptor interactions or to engagement of different signal transduction systems. On freshly isolated monocytes, Fc gamma receptor type I (FcγRI) and Fc gamma receptor type II (FcγRII) have been readily demonstrated, while the FcγRIII is present in only a subpopulation of cells or after induction by TGF-β (18, 19). The relative specificity of FcγRI for IgG isotypes is known to be IgG3 > IgG1 > IgG4 >>> IgG2. Studies on FcγRII, however, show different binding capacities based on the receptor allotype of the individual (18, 20). Designated HR (high responder) and LR (low responder), defined by their effectiveness in a T cell proliferation assay, HR individuals exhibit a pattern of specificity of IgG3 > IgG1 >>> IgG2, IgG4, while LR individuals exhibit the specificity IgG3 > IgG1 = IgG2 >>> IgG4 (20). In our studies all donors ($n = 6$) consistently responded

most vigorously to IgG2 when compared to the other IgG subclasses. It seems unlikely that all donors had the same FcγRII allotype. Differential interactions of the subclasses with FcγRI and FcγRII may lead to differing cytokine responses. For example, the superior ability of IgG2 to interact with FcγRII, as compared to IgG4 (18–20), may result in its optimal stimulation of TNFα production via that receptor. To study the relative contribution of FcγRI and FcγRII, we attempted cross-linking studies using F(ab′)2 and Fab fragments against FcγRI and FcγRII, respectively, followed by a goat anti-mouse F(ab′)2 to cross-link receptors and stimulate cytokine production. However, these studies failed to elicit cytokine production. These failures may reflect a technical problem such as inadequate binding affinity, etc. Therefore, cross-linking studies could not be further pursued. The next approach was direct blocking studies. It appears that stimulation is occurring through FcγRII, since blocking that receptor negatively affects TNFα production. The contribution of FcγRI is difficult to assess since the anti-FcγRI 32.2 antibody binds distant to the ligand binding site (17) and is, thus, ineffective at blocking cytokine production. Using a system of anti-Fc receptor antibodies, it has been demonstrated that IL-8 production can be induced secondary to FcγRI cross linking in human monocytes (21). The contribution of FcγRI and FcγRII to production of cytokines by human IgG subclasses remains to be fully elucidated, however.

Another possibility for differing patterns of TNFα, IL-6, and IL-8 production could be disparate signal transduction pathways set in motion by individual IgG subclasses. The intracellular signals that result from Fc receptor stimulation are only vaguely defined. It is known that the THP-1 cell line and freshly isolated human monocytes are stimulated via FcγRI and FcγRII, resulting in increased calcium movement and phosphorylation of intracellular proteins (22–26). In THP-1 cells it appears that cross linking of FcγRI or FcγRII results in a similar pattern of protein phosphorylation, including phospholipase C (PLC) γ1, PLCγ2, Vav, GAP and the protein kinase Syk (24, 26). The association of Syk with FcγRII or with the gamma chain of the FcγRI complex may be responsible for further phosphorylation (24, 27). The complete pathways have yet to be understood. The possibility exists that different signaling pathways are set in motion by interaction of different IgG subclasses with Fc receptors. Alternatively, promoters for the TNFα and IL-8 genes may have different sensitivity to the signaling messages produced.

One more aspect of stimulation to be considered is the interaction of various cell types in the PBMC preparation. The production of IL-8 from human monocytes versus PBMC in response to immobilized IgG has been studied (21) and confirms another study (28) in which increased IL-8 from PBMC was found as compared to production by purified monocytes. It has been suggested that two distinct pathways operate for IL-8 stimulation in monocytes. The first pathway occurs through stimulation of monocyte FcγRI using an immobilized anti-

FcγRI antibody. The second pathway is triggered by FcγRIII stimulation of lymphocytes, resulting in production of a soluble, monocyte-stimulating factor (21). Since our PBMC contain both T cells and monocytes, this second pathway may explain the ability of all IgG subclasses to stimulate IL-8 production. While the subclasses may differ in their ability to stimulate monocytes directly via Fc receptors, they may all affect a second, possibly more sensitive, lymphocyte pathway equally well. These and other questions will need to be answered to complete our understanding of IgG subclass and Fc receptor interactions.

The clinical applications of our findings remain to be determined. It is well known that different classes of immunoglobulins demonstrate deposition in human tissues expressing inflammatory responses (e.g., predominance of IgG and IgM in vascular and glomerular deposits of patients with systemic lupus erythematosus, IgA deposits in vessels and glomeruli of patients with Henoch-Schonlein vasculitis, etc.). Much less is known about IgG subclass deposition, although there are some interesting correlations. In systemic lupus erythematosus patients, it has been found that the presence of certain subclasses of antibodies correlate with distinct clinical features (11). IgG3 anti-Sm antibodies correlate with joint involvement while IgG4 anti-U1 ribonucleoprotein antibodies significantly correlate with muscle involvement. In glomerulonephritis, IgG3 is the most prevalent IgG subclass in each form of the disease (29). In patients with membranous glomerulonephropathy, IgG4 is the second most abundant subclass, a striking finding since IgG4 normally constitutes only approximately 4% of IgG in the serum. As for the clinical role of cytokines and IgG subclasses, it has been known for over a decade that children and adults lacking or deficient in IgG2 often suffer from recurrent pyrogenic infections (5, 30). Further, it has been shown that treatment which increases serum IgG2 (such as following intravenous immunoglobulin therapy) can significantly decrease the incidence of bacterial infections (31, 32). The possibility exists that either the opsonin content of the infused IgG2 or enhanced cytokine production by IgG2 may account for the protective effects of intravenously infused immunoglobulins. In some experimental models of infection, TNFα has been shown to be a critical part of host resistance. Blocking the endogenous TNFα response can lead to worsening of the infection or death of the animal (33–35). These concepts fit well with the idea of IgG2 as a potent inducer of TNFα and may be a factor in resistance to bacterial infections. While the physiological role of the IgG subclasses is not currently understood, the fact that cytokine responses of PBMC appear to depend on the class and subclass of Ig may be predictive of pathophysiological associations.

Acknowledgments—This work is supported in part by NIH grants HL-31963, AI-07413, GM-07863 and GM-50401. The authors would like to thank Pamela Lincoln for her technical assistance and Beverly Schumann for her assistance in the preparation of the manuscript.

REFERENCES

1. DRAY, S. 1960. Three gamma-globulins in norman serum revealed by monkey precipitins. *Science* **132:**1313–1320.
2. TERRY, W. D., and J. L. FAHEY. 1964. Subclasses of human gamma 2-globulin based on differences in the heavy polypeptide chains. *Science* **146:**400 408.
3. GREY, H. M., and H. G. KUNKEL. 1964. H chain subgroups of myeloma proteins and normal 72 gamma-globulin. *J. Exp. Med.* **120:**253–260.
4. SCHUR, P. H. 1987. IgG subclasses - A review. *Annals of Allergy* **58:**89–96.
5. SIBER, G. R., P. H. SCHUR, A. C. AISENBURG, S. A. WEITZMAN, and G. SCHIFFMAN. 1980. Correlation between serum IgG2 concentrations and the antibody response to bacterial polysaccharide antigens. *NEJM* **303:**178–182.
6. HEINER, D. C. 1984. Significance of Immunoglobulin G Subclasses. *Am. J. Med.* **76:**1–6.
7. URBANEK, R. 1988. IgG subclasses and subclass distribution in allergic disorders. *In* Monographs in Allergy, Vol. 23. F. Skavril, A. Morell, B. Perret, editors. Karger, Basel. CH. 33–40.
8. NUSSLEIN, H. G., M. WINTER, T. TRAG, and J. R. KALDEN. 1991. Interleukin-4-induced IgG subclass and IgE secretion by mononuclear cells from atopic donors. *Int. Arch. Allergy Appl. Immunol.* **95:**257–260.
9. PHILIPSEN, E. K., S. BONDESEN, J. ANDERSEN, and S. LARSEN. 1995. Serum immunoglobulin G subclasses in patients with ulcerative colitis and Crohn's disease of different disease activities. *Scan. J. of Gastroenterol.* **30(1):**50–53.
10. SCOTT, M. G., M. H. NAHM, G. S. NASH, M. J. BERTOVICH, and R. P. MACDERMOTT. 1986. Spontaneous secretion of IgG subclasses by intestinal mononuclear cells: differences between ulcerative colitis, Crohn's disease, and controls. *Clin. Exp. Immunol.* **66:**209–215.
11. TOKANO, Y., M. YASUMA, S. HARADA, Y. TAKASAKI, H. HASHIMOTO, K. OKUMURA, and S. HIROSE. 1991. Clinical significance of IgG subclasses of anti-Sm and U1 ribonucleoprotein antibodies in patients with systemic lupus erythematosus and mixed connective tissue disorders. *J. of Clin. Immunol.* **11:**317–325.
12. PATRY, C., A. HERBELIN, A. LEHUEN, J. F. BACH, and R. C. MONTEIRO. 1995. Fcα receptors mediate release of tumor necrosis factor-α and interleukin-6 by human monocytes following receptor aggregation. *Immunol.* **86:**1–5.
13. DEFORGE, L. E., A. M. PRESTON, E. TAKEUCHI, J. KENNEY, L. A. BOXER, and D. G. REMICK. 1993. Regulation of interleukin 8 gene expression by oxidant stress. *J. Biol. Chem.* **268:**25568–25576.
14. DEFORGE, L. E., and D. G. REMICK. 1991. Sandwich ELISA for detection of picogram quantities of IL-8. *Immunol. Invest.* **20:**89–97.
15. AARDEN, L., P. LANSDROP, and E. DEGROOT. 1985. A growth factor for B cell hybridomas produced by human monocytes. *Lymphokines* **1:**175–185.
16. EVANOFF, H., M. D. BURDICK, S. A. MOORE, S. L. KUNKEL, and R. M. STRIETER. 1992. A sensitive ELISA for the detection of human monocyte chemoattractant protein-1 (MCP-1). *Immunol. Invest.* **21:**39–45.
17. SHEN, L., P. M. GUYRE, C. L. ANDERSON, and M. W. FANGER. 1986. Heteroantibody-mediated cytotoxicity: Antibody to the high affinity Fc receptor for IgG mediated cytotoxicity by human monocytes that is enhanced by interferon-γ and is not blocked by human IgG. *J. of Immunol.* **137:**3378–3382.
18. VAN DE WINKEL, J. G. J., and C. L. ANDERSON. 1991. Biology of human immunoglobulin G Fc receptors. *J. of Leukoc. Biol.* **49:**511–524.
19. WALLACE, P. K., A. L. HOWELL, and M. W. FANGER. 1994. Role of Fcγ receptors in cancer and infectious disease. *J. Leukoc. Biol.* **55:**816–826.

20. VAN DE WINKEL, J. G. J., and P. J. A. CAPEL. 1993. Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. *Immunol. Today.* **14**:215–221.
21. MARSH, C. B., C. L. ANDERSON, M. L. LOWE, and M. D. WEWERS. 1996. Monocyte IL-8 release is induced by two independent FcγR-mediated pathways. *J. Immunol.* **157**:2632–2637.
22. SCHOLL, P. R., D. AHERN, and R. S. GEHA. 1992. Protein tyrosine phosphorylation induced via the IgG receptors FcγRI and FcγRII in the human monocytic cell line THP-1. *J. Immunol.* **149**:1751–1757.
23. CORVAIA, N., I. G. REISCHL, E. KROEMER, and G. C. MUDDE. 1995. Modulation of Fcγ receptor-mediated early events by the tyrosine phosphatase CD45 in primary human monocytes. Consequences for interleukin-6 production. *Eur. J. Immunol.* **25**:738–744.
24. GHAZIZADEH, S., J. B. BOLEN, and H. B. FLEIT. 1995. Tyrosine phosphorylation and association of Syk with FcγRII in monocytic THP-1 cells. *Biochem. J.* **305**:669–674.
25. RANKIN, B. M., S. A. YOCUM, R. S. MITTLER, and P. A. KIENER. 1993. Stimulation of tyrosine phosphorylation and calcium mobilization by Fcγ receptor cross-linking. *J. Immunol.* **150**:605–616.
26. KIENER, P. A., B. M. RANKIN, A. L. BURKHARDT, G. L. SCHIEVEN, L. K. GILLILAND, R. B. ROWLEY, J. B. BOLEN, and J. A. LEDBETTER. 1993. Cross-linking of Fcγ receptor I (FcγRI) and receptor II (FcγRII) on monocytic cells activates a signal transduction pathway common to both Fc receptors that involves the stimulation of p72 Syk protein tyrosine kinase. *J. Biol. Chem.* **268**:24442–24448.
27. DURDEN, D. L., and Y. LIU. 1994. Protein-tyrosine kinase $p72^{syk}$ in FcγRI signaling. *Blood* **84**:2102–2108.
28. RUIZ DE SOUZA, V., M. P. CARRENO, S. V. KAVERI, A. LEDUR, H. SADEGHI, J. CAVAILLON, M. D. KAZATCHKINE, and N. HAEFFNER-CAVAILLON. 1995. Selective induction of interleukin-1 receptor antagonist and interleukin-8 in human monocytes by normal polyspecific IgG (intravenous immunoglobulin). *Eur. J. Immunol.* **25**:1267–1273.
29. BANNISTER, K. M., G. S. HOWARTH, A. R. CLARKSON, and A. J. WOODROFFE. 1983. Glomerular IgG subclass distribution in human glomerulonephritis. *Clinical Nephrology* **19**:161–165.
30. OXELIUS, V. A. 1984. Immunoglobulin G (IgG) subclasses and human disease. *Am. J. Med.* **76(3A)**:7–18.
31. SILK, H. J., D. AMBROSINO, and R. S. GEHA. 1990. Effect of intravenous gammaglobulin therapy in IgG2 deficient and IgG2 sufficient children with recurrent infections and poor response to immunization with *Hemophilus influenzae* Type b capsular polysaccharide antigen. *Annals of Allergy.* **64**:21–25.
32. ANNEREN, G., C. G. M. MAGNUSSON, and S. L. NORDVALL. 1990. Increase in serum concentrations of IgG2 and IgG4 by selenium supplementation in children with Down's syndrome. *Arch. Dis. Children* **65**:1353–1355.
33. NAKANE, A., T. MINAGAWA, and K. KATO. 1988. Endogenous tumor necrosis factor (cachectin) is essential to host resistance against Listeria monocytogenes infection. *Infect. Immun.* **56**:2563–2569.
34. HAVELL, E. A. 1989. Evidence that tumor necrosis factor has an important role in antibacterial resistance. *J. Immunol.* **143**:2894–2899.
35. ECHTENACHER, B., W. FALK, D. N. MANNEL, and P. H. KRAMMER. 1990. Requirement of endogenous tumor necrosis factor/cachectin for recovery from experimental peritonitis. *J. Immunol.* **145**:3762–3766.