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Fusobacterium nucleatum Inhibits Human T-Cell Activation by Arresting Cells in the Mid-G_1 Phase of the Cell Cycle

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Fusobacterium nucleatum **has been implicated in the pathogenesis of several diseases, including urinary tract infections, bacteremia, pericarditis, otitis media, and disorders of the oral cavity such as pulpal infections, alveolar bone abscesses, and periodontal disease. We have previously demonstrated that sonic extracts of *F. nucleatum* FDC 364 were capable of inhibiting human T-cell responses to mitogens and antigens. In this study, we have further characterized this immunosuppressive protein (FIP) and initiated experiments to determine its mode of action. The purified FIP has an apparent molecular mass of 90 to 100 kDa; sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicates that the FIP is actually composed of two subunits with molecular masses of 48 and 44 kDa. Purified FIP retained its biological activity and was capable of inhibiting mitogen-induced proliferation of human T cells. Inhibition was dose dependent, and the FIP exhibited a specific activity approximately 250-fold greater than that of the crude extract. Cell cycle analysis indicates that FIP-treated cells were prevented from exiting the G_0/G_1 phase of the cell cycle. However, FIP did not alter the expression of activation markers (CD69, CD25, and CD71) or interleukin-2 secretion. The latter observations suggest that the T cells did indeed become activated and had entered the G_1 phase of the cell cycle. Analysis of the expression of cyclins indicates that the phase of the cell cycle that is FIP sensitive resides somewhere beyond the restriction point of cyclin D2 (early to mid-G_1) but prior to that of cyclins D3 and E (mid- to late G_1). Finally, analysis of the expression of the proliferating cell nuclear antigen indicates that this is the earliest detectable defect in T cells exposed to FIP. We propose that if a block in the G_1 phase of the cell cycle occurs in vivo in lymphocytes, it may result in a state of local and/or systemic immunosuppression. These suppressive effects could alter the nature and consequences of host-parasite interactions, thereby enhancing the pathogenicity of *F. nucleatum* itself or that of some other opportunistic organisms.**

Fusobacterium nucleatum is a gram-negative, anaerobic organism that has been implicated as an opportunistic pathogen in several diseases. This species has been observed as the sole infecting organism in several cases of bacteremia (5), urinary tract infection (19), and pericarditis (32). In addition, *F. nucleatum* has been implicated in several pediatric diseases, including upper respiratory tract infections and otitis media (4). Furthermore, organisms such as *F. nucleatum* have been reported to act as cofactors for diseases such as Burkitt's lymphoma and nasopharyngeal sarcoma because of their ability to produce metabolic products that may act as inducers of latent viruses such as Epstein-Barr virus (8). Finally, *F. nucleatum* is a suspected pathogen in several oral diseases such as pulp infections, alveolar abscesses, and periodontal disease (21, 29, 33, 34). The importance of *F. nucleatum* in oral disease is of particular significance because the oral route is the suspected portal of entry of this organism in other diseases.

In spite of its implication in numerous disease states, there is little information available regarding how *F. nucleatum* might act to cause disease. In this regard, we have previously demonstrated that soluble cytoplasmic extracts of *F. nucleatum* FDC 364 can suppress human lymphocyte proliferative responses to both mitogens and antigens in vitro (26). Suppression was observed to be dose dependent and to occur by noncytotoxic mechanisms and involved alterations in DNA, RNA, and protein synthesis. We have now purified the protein responsible for these immunosuppressive effects and have also determined the mechanism by which this agent acts. Our results indicate that the *F. nucleatum* inhibitory protein (FIP) arrests human T cells in the middle of the G_1 phase of the cell cycle. Our results do not provide direct evidence that immunosuppression occurs in vivo. Nevertheless, it is reasonable to propose that if this organism acts in vivo as it does in vitro, inhibition of the immune response could result in the enhanced pathogenicity of *F. nucleatum* itself or that of other opportunistic organisms.

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MATERIALS AND METHODS

Purification of FIP. FIP was prepared from *F. nucleatum* FDC 364 as previously described (26). Briefly, the bacteria were grown for 3 to 4 days at 37°C in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) supplemented with 0.2% yeast extract (Difco), 0.5 mg of L-cysteine hydrochloride, and freshly prepared 0.5% sodium bicarbonate in a hydrogen-carbon dioxide atmosphere. The soluble protein fraction was isolated from bacterial cells that were harvested, washed in phosphate-buffered saline (PBS), and disrupted by sonication at 4°C. Unbroken cells were removed by centrifugation at 12,000 × *g* for 20 min, and the membrane fraction was sedimented by centrifugation at 85,000 × *g* for 60 min. The protein that remained in suspension after the high-speed centrifugation was designated the cytoplasmic fraction and contained both cytoplasmic and periplasmic proteins. The cytoplasmic fraction was first fractionated by ammonium sulfate precipitation; all activity precipitated between 60 and 80% $(NH_4)_2SO_4$. Following dialysis against 10 mM Tris buffer, pH 7.0, containing 10 mM NaCl and 1 mM EDTA, the sample was applied to an ion-exchange column (Mono Q10; Pharmacia, Uppsala, Sweden) preequilibrated in this buffer. The column was then extensively washed and eluted with a linear NaCl gradient (10 to 600 mM). Fractions were collected and monitored for both A_{280} and FIP activity (reduction of mitogen-induced [^{3}H]thymidine incorporation) (26). FIP activity is expressed in ID_{50} units per milliliter, which represent the volume of the sample required to reduce [^{3}H]thymidine incorporation (see below) to 50% of control values. Active fractions from ion-exchange chromatography were pooled, dialyzed, and fractionated for a second time by ion-exchange chromatography

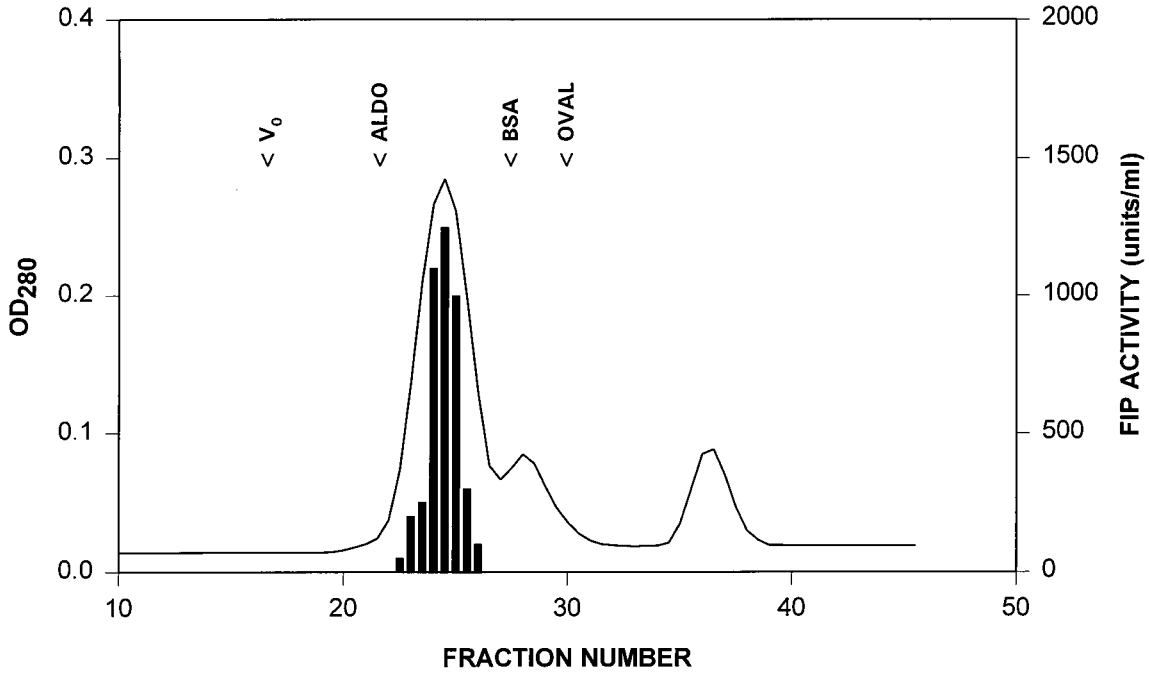

FIG. 1. Gel filtration chromatography of soluble extracts of *F. nucleatum* FDC 364. FIP was fractionated by ion-exchange chromatography as described in Materials and Methods. Active fractions were pooled, concentrated, and then further fractionated on a Superose 12 column. The optical density profile at 280 nm (OD_{280}; solid line) along with molecular weight markers are shown. V_0, void volume; ALDO, aldolase; BSA, bovine serum albumin; OVAL, ovalbumin. Fractions were assayed for their ability to inhibit T-cell proliferation (bars); results indicate the amount of FIP activity (ID_{50} units per milliliter) in each fraction.

(Mono Q5 column; Pharmacia). Active fractions were pooled and concentrated for further fractionation by gel filtration chromatography on a Superose 12 column (Pharmacia). All fractions were monitored for A_{280} and FIP activity. The active fractions were pooled, concentrated by ultrafiltration, and further fractionated by electrophoresis in 10% acrylamide gels under nondenaturing conditions. The gel was sliced into equal sections, and FIP was then eluted from the gel for 24 h in 100 mM sodium acetate containing 0.5% Nonidet P-40. Preparations were routinely assessed for purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and protein concentration was determined (BCA protein assay; Pierce, Rockford, Ill.), using bovine serum albumin as a standard.

Cell preparation and proliferation assays. Human peripheral blood mononuclear cells (HPBMC) were prepared as described previously (26). Briefly, HPBMC were isolated from 100 to 200 ml of heparinized venous blood obtained from healthy donors. The blood was diluted with an equal volume of Hanks balanced salt solution, and the HPBMC were isolated by buoyant density centrifugation on Ficoll-Hypaque (Pharmacia). The HPBMC were washed twice with RPMI 1640, and viable-cell counts were performed by assessing trypan blue dye exclusion.

An HPBMC suspension (0.1 ml) containing 2×10^5 cells was placed into each well of flat-bottomed microculture plates. Each culture received 0.1 ml of medium or 0.1 ml of various concentrations of FIP diluted in medium. The cells were then incubated for 30 min at 37°C, at which time the cell cultures received an optimal mitogenic dose of phytohemagglutinin (PHA) (1 μg/ml; Murex Diagnostics; Atlanta, Ga.). The cells were incubated for 96 h, and DNA synthesis was assessed by the incorporation of [^{3}H]thymidine as previously described (26).

Fluorescence-activated cell sorter (FACS) analysis of activation markers. HPBMC (5×10^6/ml) were incubated for 24 h with PHA in the presence or absence of FIP. The cells were washed three times with PBS containing 2% fetal bovine serum and stained with anti-CD25, anti-CD69, or anti-CD71 monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC) (Becton Dickinson Immunocytometry Systems, San Jose, Calif.). The cells were fixed in 1% paraformaldehyde and analyzed on a Becton Dickinson FacStar^{PLUS} flow cytometer (27). Appropriate isotype-conjugated control antibodies were employed (Becton Dickinson Immunocytometry Systems). Cells which gave fluorescence signals brighter than that observed for 98% of appropriate control cells were considered to be positive; 10,000 cells were analyzed per sample.

Cell cycle analysis. Cell cycle analysis was performed on HPBMC by a modification of the method of Schmid et al. (22). Briefly, 1-ml cultures (2×10^6 cells) were incubated for 24 to 72 h in the presence of medium (control), PHA, or PHA and FIP. Cells were harvested at 24-h intervals, washed (PBS containing 0.1% sodium azide), and then stained with anti-CD3 (or control antibody) conjugated to FITC. The cells were washed and fixed for 60 min with cold 0.25% paraformaldehyde. After being washed, the cells were permeabilized with 0.2% Tween 20 in PBS for 15 min at 37°C. DNA was then stained by incubating cells with 7-amino-actinomycin (25 μg/ml) (7-AAD) for 30 min. Samples were analyzed on a Becton-Dickinson Facstar^{PLUS} flow cytometer equipped with pulse processing capability for doublet discrimination. FITC and 7-AAD fluorescence were excited by an argon laser operating at 488 nm, and fluorescence was measured with a 530/30-nm bandpass filter and a 650-nm-long pass filter, respectively. A minimum of 50,000 events were collected for each sample. Immunofluorescence was detected using log amplification, and 7-AAD emissions were collected using linear amplification; data were analyzed using appropriate software (Modfit; Verity Software House, Lewiston, Maine).

Analysis of expression of cell cyclins and PCNA. HPBMC (2.5×10^6) were incubated with PHA in the presence or absence of FIP for 24 to 72 h. The cells were harvested and solubilized in 50 mM Tris containing 0.5% Nonidet P-40, 150 mM NaCl, 1 mM sodium vanadate, 50 mM sodium fluoride, 1 mM benzamidine, and 25 μg each of aprotinin, leupeptin, and trypsin inhibitor per ml (Sigma Chemical Co., St. Louis, Mo.). Cell debris was removed by centrifugation (10,000 $\times$ *g*). Proteins of interest were detected by Western blot (immunoblot) analysis using monoclonal antibodies to the individual cyclins (D2, D3, E, B, and A) and proliferating cell nuclear antigen (PCNA) (Pharmingen, San Diego, Calif.). Briefly, total solubilized cellular protein was separated by 10% SDS–PAGE and then transferred to nitrocellulose. The membrane was blocked with BLOTTO (28) and then incubated with one of the primary antibodies listed above for 18 h at 4°C. Membranes were washed, incubated with rabbit anti-mouse immunoglobulin sera conjugated to alkaline phosphatase (Fisher Scientific); the blots were developed with the chromogenic substrate 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (28).

RESULTS

We previously reported that crude sonic extracts of *F. nucleatum* FDC 364 contain a heat-labile, nondialyzable protein capable of inhibiting human T-lymphocyte activation by both mitogens and antigens (26). We have now purified this immunomodulatory protein to apparent homogeneity (see Materials and Methods for details). Based upon gel filtration chromatography (Fig. 1), FIP elutes as a single well-defined peak corresponding to a molecular mass of 90 to 100 kDa. However, SDS-PAGE analysis of the purified FIP revealed the presence of two peptides with molecular masses of 48 and 44 kDa (Fig.

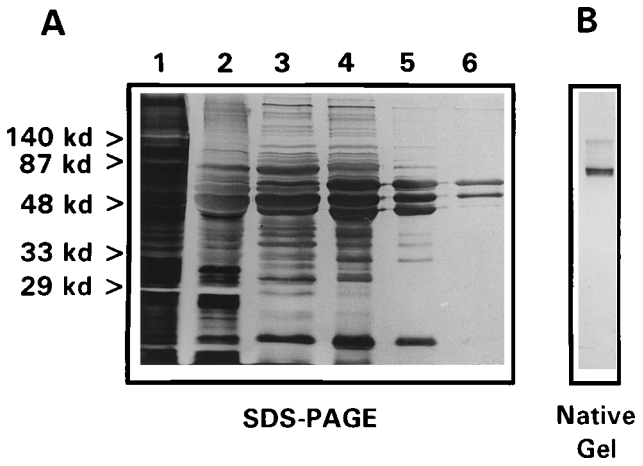

FIG. 2. SDS-PAGE of FIP isolated from *F. nucleatum* FDC 364. FIP was purified as described in Materials and Methods. (A) SDS-PAGE of samples from different stages of FIP purification. Lane 1, crude SE; lane 2, 60 to 80% ammonium sulfate pool; lane 3, Mono Q10 pool; lane 4, Mono Q5 pool; lane 5, Superose 12 pool; lane 6, material eluted from native gel. (B) Purified FIP electrophoresed on a native, nondenaturing gel. Note: the molecular mass markers apply only to the SDS-PAGE.

2A). The two peptides appear to be present in approximately equivalent amounts, suggesting that the native FIP may exist as a heterodimer. The existence of a subunit structure was further supported by analysis of FIP on native (nondenaturing) gels, where the protein ran as a single band (Fig. 2B).

The purified FIP retained its biological activity. Human T cells exposed to various amounts of FIP (0.025 to 1.0 μg of protein per ml) exhibited a dose-dependent reduction in their ability to proliferate in response to PHA (Fig. 3). Inhibition was also observed for other mitogens such as phorbol myristate acetate, ionomycin, and concanavalin A ConA; the proliferative responses of purified preparations of B cells were also inhibited (data not shown). Purified preparations of FIP typically exhibited an ID_{50} (dose required to cause 50% inhibition of [^{3}H]thymidine incorporation) of 0.17 μg of protein per ml. In comparison, the crude FIP extracts exhibited an ID_{50} of 45.1 μg of protein per ml; this represents a >250-fold increase in specific activity.

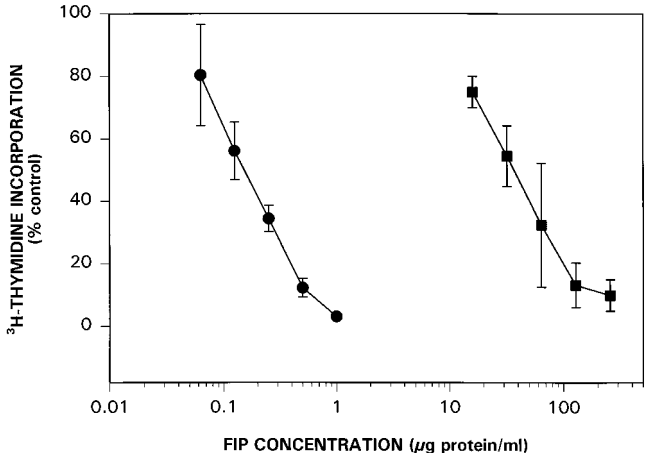

FIG. 3. Comparison of effects of crude and pure FIP on HPBMC proliferation. HPBMC were incubated with various amounts of either crude (squares) or purified (circles) FIP for 30 min; an optimal mitogenic dose of PHA was then added. [^{3}H]thymidine incorporation was measured after incubation for 4 days. The results are plotted as the percentage of [^{3}H]thymidine incorporation in control cultures receiving mitogen alone. Each point represents the mean ± standard error of three experiments, each performed in quadruplicate. [^{3}H]thymidine incorporation averaged 460 cpm in control cultures receiving medium alone and 41,722 cpm in control cultures exposed to PHA.

TABLE 1. Effect of FIP on PHA-induced cell cycle progression in T cells

Cell treatment	Cell cycle distribution[a] (%)		
	G_0/G_1	S	G_2/M
None (cells only)	95.7 ± 1.2	3.5 ± 1.0	0.8 ± 0.2
PHA only	48.1 ± 3.9	44.3 ± 5.5	10.7 ± 3.8
PHA + FIP (0.10 μg/ml)	69.0 ± 4.7[b]	24.4 ± 4.1[b]	7.2 ± 1.0
PHA + FIP (0.25 μg/ml)	75.0 ± 10.0[b]	20.9 ± 9.4[b]	4.1 ± 1.5
PHA + FIP (0.50 μg/ml)	85.7 ± 8.0[b]	11.5 ± 7.2[b]	2.4 ± 0.5

[a] T cells were incubated with medium or PHA in the absence or presence of various amounts of FIP for 72 h. Cells were then stained with anti-CD3 conjugated to FITC and 7-AAD as described in Materials and Methods. Cell cycle analysis was performed by fluorescence-activated cell sorting after gating on $CD3^+$ cells. Results represent the means ± standard errors of the means of five experiments, each performed in duplicate.

[b] When compared with the PHA controls, differences in these values were found to be statistically significant ($P < 0.05$).

Failure of T cells to proliferate in the presence of FIP was further documented by cell cycle analysis. Table 1 shows the cell cycle distribution of T cells exposed to PHA in the presence or absence of FIP. As expected, unstimulated T cells were found predominantly in the G_0/G_1 phase (95.7%) of the cell cycle, whereas PHA treatment resulted in the transition of a significant portion of the T cells from the G_0/G_1 phase into the S phase (44%) as well as into the G_2 and M phases (10.7%) of the cell cycle. In contrast, cells exposed to FIP were inhibited from exiting G_0/G_1 and entering into S, G_2, and M. Inhibition of the cell cycle was also dose dependent (0.1 to 0.5 μg of protein per ml).

We next evaluated the effects of FIP on earlier stages of cell activation. Specifically, we initially focused on events associated with the transition through the G_1 phase of the cell cycle. FIP-treated cells were first analyzed for their ability to express surface antigens associated with mitogen- and antigen-induced T-cell activation. These included CD69 (activation inducer molecule), which is one of the earliest antigens to be expressed following cell activation with a mitogen or antigen; interleukin-2 (IL-2) receptor (CD25), a 55-kDa glycoprotein which is present on the surface of lymphocytes within 24 h after activation; and the transferrin receptor (CD71), which is expressed in the mid- to late G_1 phase of the cell cycle. The results of these experiments are shown in Fig. 4. Compared with control cells (no mitogen), PHA-activated T cells exhibited increased expression of CD69 (86.5%), CD25 (65.5%), and CD71 (34.4%). FIP failed to influence the percentage of cells expressing these activation markers; also, there was no reduction in the level of expression of these activation markers in FIP-treated T cells. Likewise, other events associated with the early stages of cell activation, such as cytokine production (e.g., IL-2), were not inhibited in the presence of comparable concentrations of FIP (data not shown). It should be noted that higher concentrations of FIP (e.g., 5.0 μg/ml) did cause a moderate decrease in IL-2 production.

Our experiments clearly indicate that mitogen-induced T-cell activation proceeds normally through the early cascade of events in the presence of FIP, i.e., transition from G_0 to G_1. Therefore, we next conducted experiments to determine if FIP blocked later events associated with the transition of lympho-

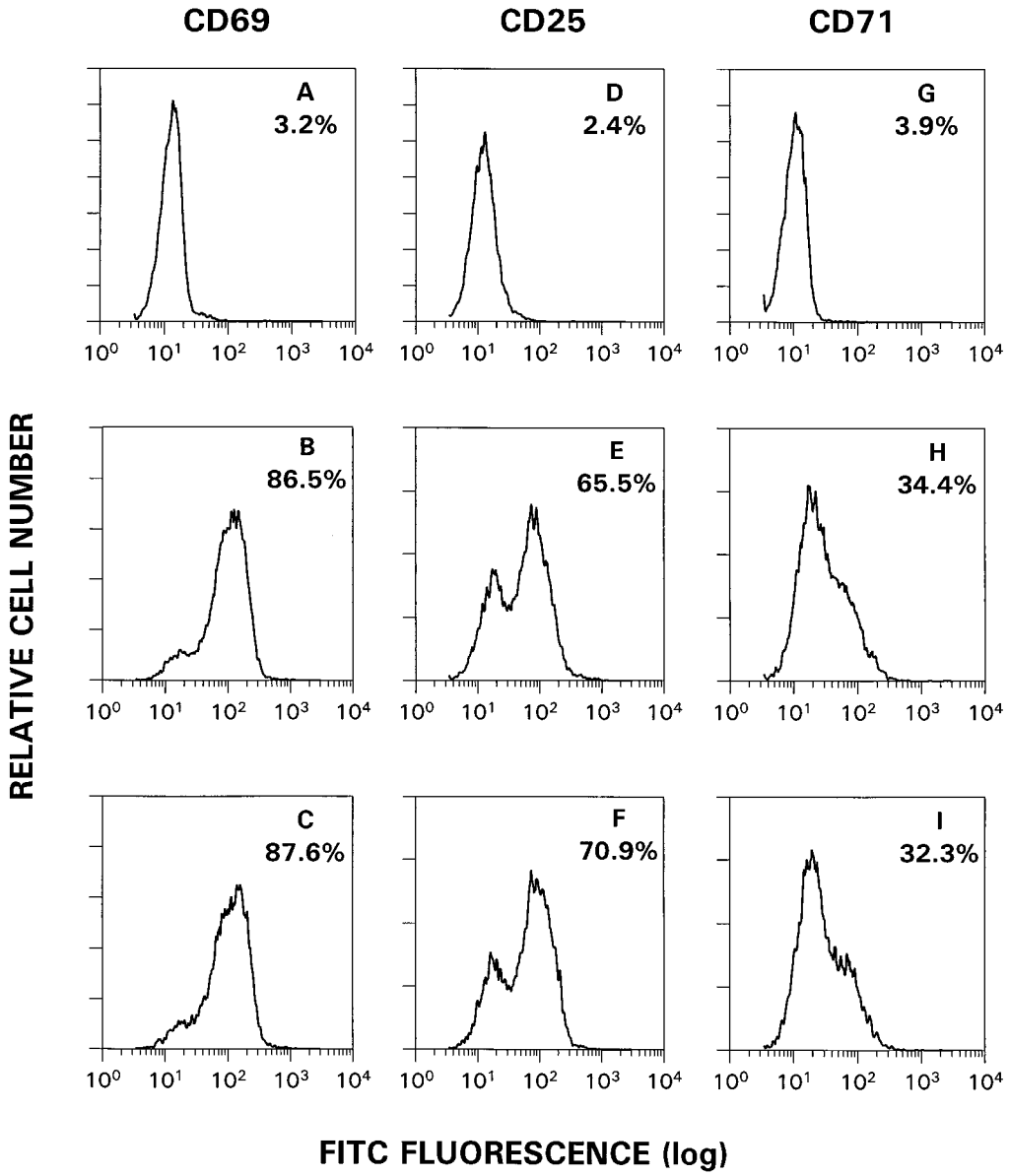

FIG. 4. Effect of FIP on expression of activation markers in T cells. T cells were incubated with medium (A, D, and G), PHA (B, E, and H), or PHA and 0.5 μg of FIP per ml (panels C, F, and I) for 24 h, and the cells were stained as described in Materials and Methods. Results are displayed for each marker (CD69, CD25, and CD71). The numbers in each panel represent the percentage of positive cells; analysis gates were set so that ≥98% of cells stained with isotypic control antibodies remained outside the positive region. Results are representative of four experiments; at least 10,000 cells were analyzed.

cytes either through the G_1 phase of the cell cycle, or alternatively, into the S phase. FIP was analyzed for its ability to alter the expression of key elements that regulate cell cycle progression; these include the cyclins and PCNA. The levels of these proteins have been shown to vary as cells progress through the cell cycle; furthermore, they form functional complexes which in turn regulate the progression of T cells through the cell cycle. As shown in Fig. 5, resting cells did not express appreciable levels of any of these proteins. In contrast, expression of the cyclins and PCNA was induced in PHA-treated cells. Cyclins D2, D3, and E, as well as PCNA, appear within 24 h of activation, whereas cyclins A and B were not expressed at significant levels until 48 h (Fig. 5). However, a different pattern of expression was observed in T cells exposed to FIP. Cyclins A and B, which are associated with regulation of the S, G_2, and M phases of the cell cycle, were not expressed in FIP-treated T cells at any of the time intervals examined. Also, FIP-treated cells failed to express cyclin D3 and E, which are associated with the mid- to late G_1 phase. In contrast, expression of cyclin D2 (associated with the early to mid-G_1 phase) was not affected by FIP, and in some instances the levels of cyclin D2 appeared to increase in the presence of FIP. Furthermore, PCNA expression was significantly reduced, and in some instances, it was barely detectable in T cells treated with FIP. It should be noted that expression of cyclin D1 was not detected in any of the control populations or in the FIP-treated cultures.

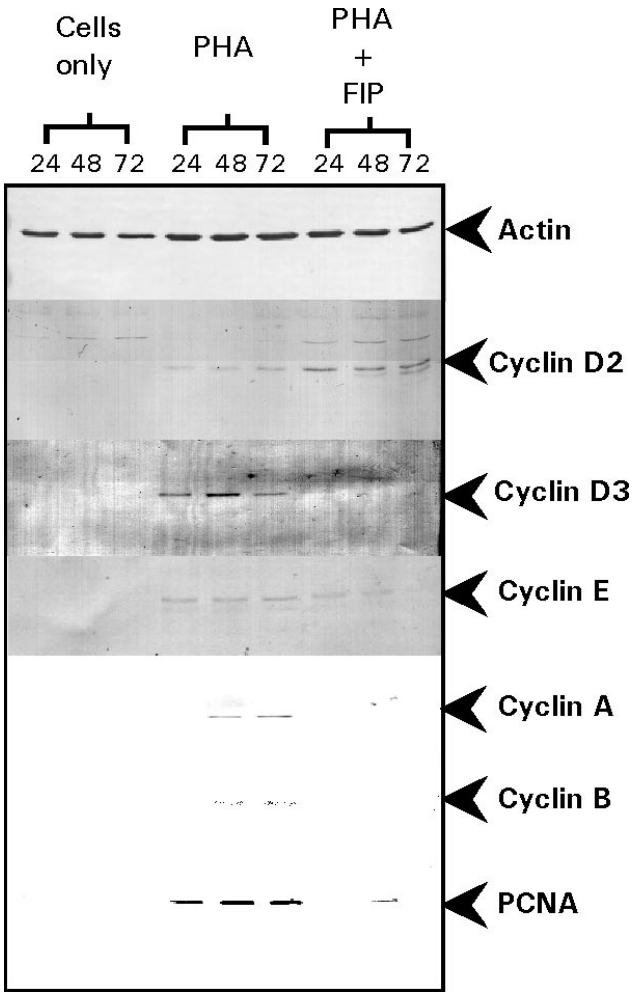

FIG. 5. Effects of FIP on expression of cyclins and PCNA. T cells were incubated in the presence of medium, PHA, or PHA and 0.5 μg of FIP per ml for 24, 48, and 72 h. Cells were harvested and disrupted, and the cell extracts were analyzed for the presence of the individual cyclins and PCNA by Western blot. Results for each cyclin (and PCNA) are shown; actin was employed to demonstrate that the same amount of sample (10 μg) was applied to each lane.

DISCUSSION

In this study, we demonstrated that purified FIP was capable of inhibiting mitogen-induced human T-cell proliferation in a dose-dependent fashion. Specifically, FIP-treated cells failed to incorporate [^{3}H]thymidine, and, furthermore, cell cycle analysis indicates that these cells remained in the G_0/G_1 phase of the cell cycle. It should be noted that the methods employed for cell cycle analysis did not enable us to discriminate between the G_0 and G_1 phases. However, results from other experiments clearly indicate that the FIP-treated cells did indeed proceed through the early stages of cell activation and enter the G_1 phase of the cell cycle. For example, T cells exposed to FIP and mitogen were capable of expressing cell surface activation markers; CD69, CD25, and CD71 were expressed at the same levels in the cells exposed to FIP as they were in the PHA control population. Likewise, these cells were also capable of producing IL-2. Expression of the activation markers and secretion of cytokines are generally associated with entry into G_1 and transition through the early to mid-G_1 phase of the cell cycle (Fig. 6) (12, 13, 15). We, therefore, focused our attention on the effects of FIP on regulatory elements of the cell cycle.

Cell cycle regulation is generally accepted to be controlled at discrete points, called checkpoints, by complexes of cyclins and a family of related protein kinases, the cyclin-dependent kinases (CDKs) (reviewed in reference 17). The cyclins are a group of proteins that undergo a cell cycle-dependent variation in level, with different cyclins playing critical roles at distinct stages of the cell cycle (17). As shown in Fig. 6, complexes of CDKs and cyclins D2, D3, and E are associated with regulation of the transition through the G_1 phase of the cell cycle. For example, cyclin D2 expression begins early in G_1, or even possibly at the G_0-to-G_1 transition (1, 7). Cyclins D3 and E are synthesized later in G_1, with maximal accumulation of cyclin E coinciding with cell entry into the S phase. On the other hand, complexes composed of CDKs and cyclins A and B regulate transition through the S, G_2, and M phases of the cell cycle (15, 17, 31). Our results indicate that cyclin D2 is expressed at normal levels in FIP-treated cells. In contrast, expression of cyclins D3, E, A, and B are inhibited in these cells. Collectively, these data indicate that the FIP-sensitive phase of the cell cycle resides somewhere beyond the restriction point of cyclin D2 but prior to that of cyclins D3 and E.

The transition of T cells from quiescence into G_1 follows a cascade of events, and as the cells progress through the G_1 phase, increases in the level of transcription of a number of other genes occurs. Of particular relevance to our study is the role of PCNA at this phase of the cell cycle. Initially, PCNA was identified as a marker for proliferating cells in the S phase. More recently it has been shown to be an auxiliary protein for DNA polymerase delta (3). Furthermore, PCNA has been shown to play a critical role in regulating cell transition through early to mid-G_1 (6). As part of this regulatory role, it is required for CDK2, CDK4, and CDK5 activation by forming a complex with the CDKs and the D-type cyclins (Fig. 6).

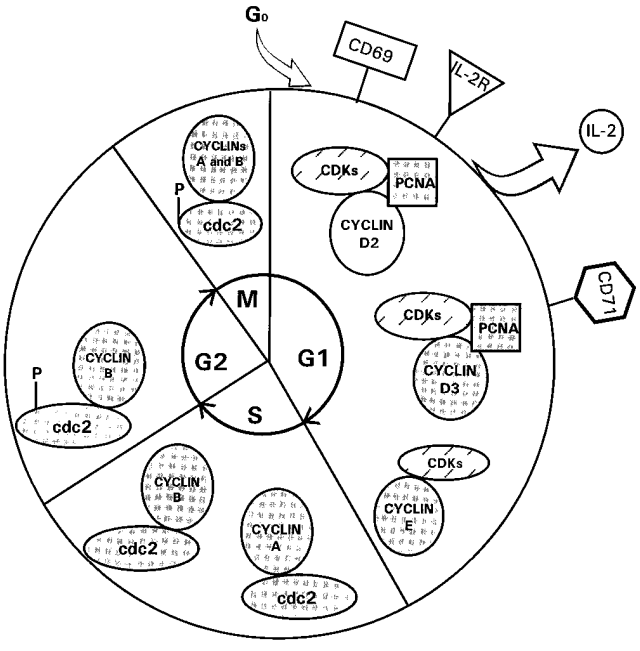

FIG. 6. Schematic diagram of the cell cycle in T cells. The relationship between cyclins, CDKs, PCNA, activation markers, and IL-2 with respect to the phase of the cell cycle is shown. Shaded symbols indicate factors known to be inhibited in FIP-treated cells; open symbols represent events not altered in these cells; hatched symbols represent events not yet analyzed. The diagram has been adapted from reference 17.

PCNA synthesis begins to increase during the early to mid-G_1 phase, approximately 12 to 15 h before entry into the S phase (30). PCNA continues to increase in amount throughout the cell cycle and remains high in proliferating cell cultures. Analysis of FIP-treated cells indicates that these T cells do not contain detectable levels of PCNA at 24 and 72 h and contain only trace levels at 48 h. This is in sharp contrast to the levels expressed in cells exposed to mitogen alone. Therefore, our results indicate that inhibition of PCNA expression represents the earliest detectable lesion in T cells exposed to FIP. Failure to produce adequate quantities of PCNA would in turn result in a cell cycle block in the mid- to late G_1 phase of the cell cycle; such a cell cycle arrest is consistent with our observations. However, it is possible that other events which occur after the cyclin D2 restriction point, but precede PCNA expression, may also be influenced by FIP. Nevertheless, the current data strongly suggest that the molecular mode of action of FIP on human T cells involves alterations in the expression of PCNA. Since the proliferative responses of purified populations of B cells were also inhibited, we believe that FIP interacts directly with lymphocytes as opposed to functioning indirectly via a regulatory cell. Experiments are being conducted to further define and directly implicate altered PCNA expression as the molecular target site for the immunoinhibitory properties of FIP.

In summary, it is well established that several infectious diseases are associated with decreased immunologic responsiveness (23). Furthermore, it has been recognized that avoidance or modulation of the immune response by invading pathogens is a critical event in determining the outcome of numerous infectious processes. These include periodontal diseases (25), measles (2), rubella (10), influenza (9), leprosy (14), leishmaniasis (18, 24), trypanosomiasis (11), syphilis (16), and candidasis (20), among others. Of particular relevance are the observations that several microorganisms are capable of suppressing the host's immune response (reviewed in reference 23). Bacterial products that may suppress immunologic responsiveness include toxins, enzymes, cell wall components, and metabolic products. These immunoregulatory agents may act via several different mechanisms that interfere with either the induction or the expression of immunity. As already mentioned, *F. nucleatum* has been implicated in the pathogenesis of several diseases and also has been considered to be an opportunistic organism infecting compromised patients. Although the immunologic mechanism(s) involved in the pathogenesis of these disorders has not been clearly defined, it is reasonable to predict that suppressed host defense mechanisms, particularly those induced by oral pathogens themselves, may contribute to the disease process. Such bacterially derived immunosuppressive factors could lead to a state of hyporesponsiveness that favors colonization by the initiating organism or by other opportunistic organisms. In conclusion, our data demonstrate that FIP disrupts the ability of T cells (and B cells) to properly transit the cell cycle, thereby causing a block during the mid-G_1 phase. This disturbance would in turn adversely affect the development of normal immunologic defense mechanisms. We propose that such immunologic perturbations could contribute to the pathogenesis of diseases associated with *F. nucleatum* infection by impairing host protection.

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