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Immunology

Eosinophil differentiation factor also has B-cell growth factor activity: Proposed name interleukin 4

(T-cell lymphokines/hemopoiesis/colony-stimulating factors)

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ABSTRACT **A mouse lymphokine that stimulates the production of functional eosinophils in liquid bone marrow cultures has recently been described [Sanderson, C. J., Warren, D. J. & Strath, M. (1985) *J. Exp. Med.* 162, 60–74]. This factor appears to be specific for the eosinophil lineage in hemopoietic differentiation and is analogous to colony-stimulating factors described for other hemopoietic lineages. In this paper we report that this factor appears to be identical to the B-cell growth factor II described by Swain and Dutton [Swain, S. L. & Dutton, R. W. (1982) *J. Exp. Med.* 156, 1821–1834]. This conclusion is based on the coordinate expression of the two activities by a panel of alloreactive T-cell clones and lines and on copurification through a series of protein separation techniques. The reason for a single lymphokine's having these widely differing biological activities is unclear, and its duality presents problems in using terminology based on either assay system. For this reason we propose the name "interleukin 4" for this molecule, and we suggest the defining property should be its eosinophil-differentiating activity.**

The study of lymphokines has evolved from testing crude lymphocyte supernatants containing a multitude of factors to the stage where well-defined preparations can be tested in well-defined assays. This has led to "convergence" as differing lymphokine activities are found to be different properties of a restricted group of molecules. For example, γ-interferon (IFN-γ) inhibits viral replication, activates macrophages, increases expression of class 2 antigens of the major histocompatibility complex and is involved in B-cell activation (1–5); interleukin 3 (IL-3) has been shown to exhibit activity in many bioassays (6), and at least a dozen names based on different assays are used for this factor (7, 8); interleukin 2 (IL-2), originally described as a T-cell growth factor (9), has been shown to have B-cell growth factor (BCGF) activity (10). These examples illustrate the difficulties that can be encountered when lymphokine names are based on assay systems.

In this report we present evidence indicating that eosinophil differentiation factor (EDF) (11), stimulates the B-cell tumor (BCL_1) cell line that is used to define BCGFII (12). We were first alerted to the possibility that these two assays may be detecting a single lymphokine when a coordinate analysis of lymphokines produced by T-cell clones revealed a highly significant correlation between eosinophil differentiation activity [EDA; i.e., activity in the assay, the main component of which is EDF (1)] and BCGFII activity. This was strengthened by the fact that various cell lines producing high levels of one of the factors were also high for the other. The possibility that a single lymphokine was involved is further suggested by biochemical separations of EDF produced by the T-cell hybrid (NIMP-TH1) that had been selected for the production of this lymphokine (13).

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MATERIALS AND METHODS

T-Cell Cloning. Alloreactive T-cell clones were isolated as described (14). Briefly, BALB/c.nimr spleen cells were cultured in microplates at limiting dilution (100 cells per well) with 2×10^5 irradiated CBA.nimr spleen cells per well and a source of IL-2. After 2 weeks in culture the clones were restimulated with irradiated T-cell-depleted CBA spleen cells in the absence of exogenous IL-2, and supernatants were collected after 48 hr.

Lymphokine Assays. All assays were carried out in microplates. IL-3 was assayed on the dependent hemopoietic cell line 32D, using the incorporation of [^{3}H]thymidine to determine cell growth (15). BCGFI was assayed by incubating the test material with 5×10^4 enriched B cells, with affinity-purified goat anti-mouse immunoglobulin at 5 μg/ml for 72 hr (16). Cell growth was assayed by adding [^{3}H]thymidine for the last 4 hr. BCGFII was assayed by incubating 1.5×10^4 BCL_1 cells per well with the test material for 48 hr (17). Growth was determined as in the BCGFI assay. EDA was assayed by incubating bone marrow cells from *Mesocestoides corti*-infected mice (10^5 per well) with test material. After 5 days eosinophils produced in the cultures were determined by assay for eosinophil peroxidase (18, 19).

Fractionation. Gel filtration was carried out on a 300 ml bed column of Ultrogel AcA 54 (LKB, Croydon, Surrey) in phosphate-buffered saline (0.15 M NaCl/0.02 M sodium phosphate, pH 7.4) containing polyethylene glycol (PEG 6000; BDH Pharmaceuticals, Poole, Dorset) at 50 μg/ml. The column was calibrated with bovine serum albumin (M_r = 66,000), ovalbumin (M_r = 45,000), carbonic anhydrase (M_r = 30,000), and cytochrome *c* (M_r = 12,400) (all from Sigma). Separation by binding to lentil lectin was carried out using LcA-Sepharose (Pharmacia, Uppsala, Sweden) and eluting with 0.1 M methyl α-mannoside. Reverse-phase HPLC was carried out on a μBondapak C_{18} column (Waters Associates). The column was eluted with acetonitrile containing 0.1% trifluoroacetic acid, from 25% to 40% (vol/vol) acetonitrile, and then from 40% to 60% acetonitrile.

RESULTS

Conditioned medium from 39 alloreactive T-cell clones were tested for their ability to stimulate eosinophil differentiation and compared with activity in the BCGFI (B-cell-stimulatory

Abbreviations: IFN-γ, γ-interferon; IL-2, interleukin 2; IL-3, interleukin 3; BCGF, B-cell growth factor; EDF, eosinophil differentiation factor; EDA, eosinophil differentiation activity (i.e., activity in the assay, the main component of which is EDF); CSF, colony-stimulating factor; TH1-CM, conditioned medium from the T-cell hybrid NIMP-TH1; T2-CM, conditioned medium from the T-cell clone NIMP-T2.

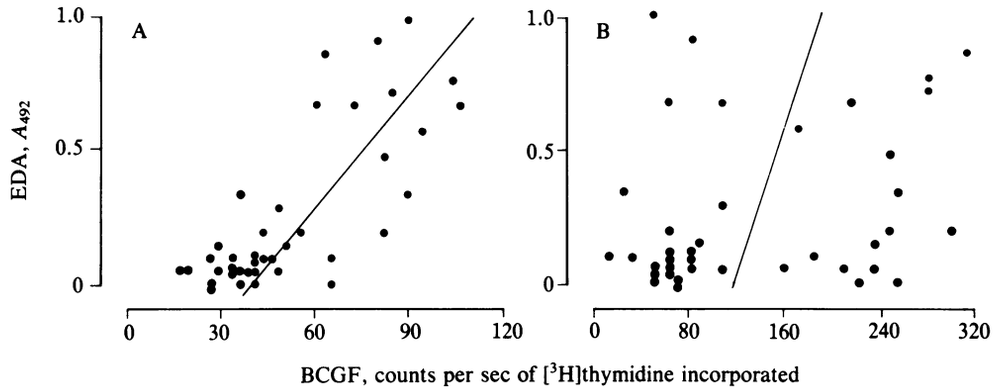

FIG. 1. Coordinate analysis of EDA and BCGFII (*A*) and EDA and BCGFI (*B*), by a panel of 39 T-cell clones. EDA is expressed as absorption units obtained in an assay for eosinophil peroxidase. Regression analysis showed the following: (*A*) $r = 0.77$, slope 6 ± 0.8, t value for slope = 7.3 ($P < 0.001$); (*B*) $r = 0.24$, slope 12.3 ± 8.1, t value for slope = 1.5 ($P > 0.05$). The line shown is the estimated best fit in each case.

factor 1, BSF-1) and BCGFII assays (Fig. 1). Regression analysis indicated that there was no significant correlation between EDA and BCGFI production ($P > 0.05$), whereas the relationship between the production of EDA and BCGFII was highly significant ($P < 0.001$). This positive correlation could indicate either that the two lymphokines were under coordinate control or that a single molecule was involved.

As shown in Table 1 the T-cell hybrid NIMP-TH1, which was known not to produce IL-2, IL-3, colony-stimulating factor (CSF), or IFN-γ (13), was found to have BCGFII activity. Another T-cell hybrid (NIM-TH12) that had been selected for BCGFII activity was found to produce high levels of EDF. In addition, two sublines of the EL4 cell line, both producing high levels of IL-2 but differing markedly in BCGFII production, were found to show a similar difference in their capacity to produce EDF. Also, the T-cell clone (NIMP-T2) maintained as a high producer of EDF (11) was a high producer of BCGFII.

These results provided circumstantial evidence that EDF and BCGFII might be properties of a single molecule, and so fractionation experiments were undertaken. First, gel filtration of NIMP-TH1 conditioned medium (TH1-CM) and NIMP-T2 conditioned medium (T2-CM) showed that in each case the EDA and BCGFII activities had similar elution patterns (Fig. 2), with a peak equivalent to M_r 46,000. IL-3, also present in T2-CM, eluted with a peak of M_r 29,000, as shown previously (11). Fractionation of TH1-CM by lentil lectin affinity chromatography, flat-bed isoelectric focusing, preparative gel electrophoresis, and elution from phenyl-Sepharose and DEAE-Sepharose showed that in each case EDF and BCGFII had identical elution profiles (data not shown). Material purified by gel filtration and then by affinity chromatography on lentil lectin was subjected to reverse-phase HPLC. The two activities coincided (Fig. 3).

Table 1. Activity of conditioned medium from different cell lines in EDA and BCGFII assays

Cell line	EDA, units	BCGFII, cpm × 10^{-3}
NIMP-TH1 (T-cell hybrid)	550	25
NIM-TH11 (T-cell hybrid)	40	9.6
NIM-TH12 (T-cell hybrid)	>1000	34
NIM-TH13 (T-cell hybrid)*	19	1.5
NIMP-T2 (T-cell clone)	>1000	38
EL4-17 (lymphoma)	50	16
EL4-23 (lymphoma)	>1000	31
Control (medium only)	0	0.5

EDA is expressed as the reciprocal of the dilution giving 50% of maximum activity. Values greater than 10 represent significant activity. BCGFII is expressed as the incorporation of [^{3}H]thymidine at a dilution of 1:10. Values above 1.5 represent significant activity (control mean + 3SD). The sublines of EL4 were kindly supplied by H. R. MacDonald. Conditioned media from EL4 cells and the T-cell hybrids were obtained after stimulation with phorbol myristate acetate or concanavalin A. Conditioned medium from NIMP-T2 was obtained after stimulation with allogeneic spleen cells (11).

*The hybrid NIM-TH13 originally showed both EDA and BCGFII activities; however, on recloning, both activities were lost simultaneously. It is included as a negative control.

DISCUSSION

The identification of EDF was aided by the construction of a T-cell hybrid (NIMP-TH1) that appeared not to produce any other lymphokines (13). This made it relatively easy to show that EDF was distinct from IL-2, IFN-γ, IL-3, and factors stimulating neutrophil and macrophage colony formation. This lymphokine is produced by T-cell clones reacting to antigens of the cestode *M. corti* as well as alloreactive T-cell clones. It stimulates the differentiation of eosinophils from

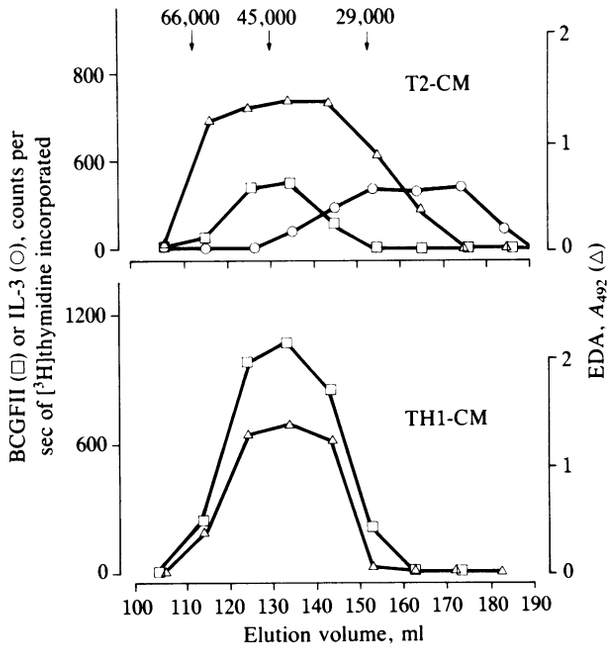

FIG. 2. Fractionation of T2-CM and TH1-CM by gel filtration on Ultrogel AcA 54. Fractions were assayed for EDA (△), BCGFII (□), and IL-3 (○). Fractions from T2-CM were tested as a final dilution of 1:100 for EDA and 1:10 for BCGFII and IL-3. Fractions from TH1-CM were tested at 1:1000 for EDA and 1:10 for BCGFII.

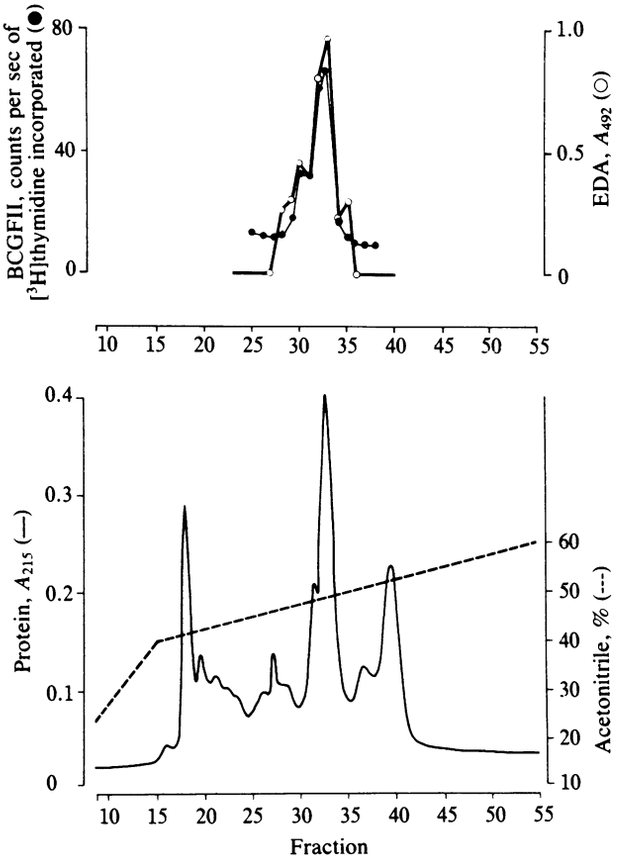

FIG. 3. Fractionation of TH1-CM by reverse-phase HPLC. The conditioned medium was first fractionated by gel filtration on Ultrogel AcA 54, and fractions high in EDA were pooled and passed through a lentil lectin column. All the EDA bound to the column was eluted with methyl α-mannoside. This material was then fractionated by HPLC. The protein profile (—) and the acetonitrile concentration in the eluting buffer (- - -) are shown in *Lower*. (*Upper*) Results of assay for EDF (○) at a dilution of 1:5000 and assay for BCGFII (●) at a dilution of 1:1000.

undifferentiated bone marrow precursor cells in liquid culture. These culture-derived eosinophils have similar levels of eosinophil peroxidase and express receptors for the same range of immunoglobulin isotypes as eosinophils taken from mice. They have the capacity to kill antibody- and complement-coated schistosomula of *Schistosoma mansoni* (11). These results indicate that EDF controls differentiation to fully mature and activated eosinophils. Furthermore, purified EDF is also active on human cells in a lineage-specific way, inducing proliferation, differentiation, and activation of eosinophils, with no detectable effect on neutrophils (unpublished data).

On the other hand, BCGFII was originally described as an activity (DL-BCGF), produced by an alloreactive T-cell line, which could be assayed on normal B cells and BCL_1 tumor cells (12). Subsequently a similar activity was partially purified from media conditioned by the lymphoma cell line EL4 (EL4-BCGFII) with M_r (gel filtration) of 55,000 (17). This factor is distinct from a lower molecular weight factor (16) also produced by EL4 cells (BCGFI, EL4-BCGFI, BSF-1).

As part of a series of experiments designed to study the expression of multiple lymphokines by T cells a coordinate analysis of the production of lymphokines by T-cell clones was carried out. There was no correlation between EDA and IL-2, IL-3, CSF, or IFN-γ (20). However, there was a high correlation between EDA and BCGFII (Fig. 1). This, together with the results showing that the two activities copurify (Figs. 2 and 3), strongly suggests that the activities are the property of a single species of molecule. Although the M_r estimate of 55,000 for BCGFII from EL4 (17) is higher than the value obtained in this paper from TH1-CM and T2-CM (45,000) the elution profile is wide, suggesting some heterogeneity. Furthermore, our studies with EL4 supernatants indicate that EDF and BCGFII from this source co-elute with mean M_r of 45,000 (unpublished data). Thus it seems likely that the disparity can be attributed to technical differences. Complete purification of EDF will allow more accurate estimates of molecular size.

Although in this work the ability to stimulate DNA synthesis in BCL_1 cells has been used as an assay for BCGFII activity, other experiments with semi-purified material from TH1-CM showed that EDF induces DNA synthesis in large B cells (preactivated *in vitro*) and in B cells activated by anti-immunoglobulin *in vitro* (unpublished data). These experiments suggest that this factor acts on B cells at a late stage in G_1 phase and confirm a direct activity on B cells rather than simply a fortuitous stimulation of the BCL_1 tumor cell line by EDF.

The biological role for these divergent activities residing in one lymphokine can only be speculated upon at the present time. It is interesting to recall that eosinophilia is frequently associated with high levels of IgE antibody, which raises the possibility that this association may be linked to the different biological activities of this lymphokine.

This work raises the problem of nomenclature for this lymphokine. The term "IL-2" has been readily accepted for the factor originally defined as a T-cell growth factor, and the term "IL-3" is gaining acceptance for what has been a confusing array of functional names. Indeed, the naming of EDF is complicated by the fact that IL-3 also has activity in this assay (11). For these reasons we propose the name "interleukin 4" for the factor we originally described as EDF (11). The eosinophil-differentiating property appears to be the most appropriate defining characteristic at the present time. Functionally active eosinophils are produced *in vitro*, it can be detected in the serum of mice with eosinophilia (19), and it induces an increased number of eosinophils in the bone marrow when injected into normal mice (unpublished data). On the other hand, the role of its BCGF activity is not as well defined.

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