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Supplementary information is available on Nature's World-Wide Web site (<http://www.nature.com>) or as paper copy from the London editorial office of Nature.

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Correspondence and requests for materials should be addressed to R.G.B. (e-mail: brennanr@ohsu.edu). Coordinates for the BmrR–TPP–DNA and BmrR–TPSb–DNA complexes have been deposited in the RCSB Protein database under accession code *lexj* and *lexi*, respectively.

correction

Halocarbons produced by natural oxidation processes during degradation of organic matter

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Nature **403**, 298–301 (2000).

Throughout Figs 2–4 and the corresponding legends, mM should have been mmol, μ M should have been μ mol, and pM should have been pmol. In addition, in Fig. 3a the *x* axis should have been labelled 'Fe (II) conc. in medium (μ mol)'; and in Fig. 3b the *x* axis should have been labelled 'Halide ion conc. in medium (mmol)'. □

erratum

Memory B-cell persistence is independent of persisting immunizing antigen

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Nature **407**, 636–642 (2000).

In this paper, Table 1 was printed incorrectly. It should have appeared as below. □

Table 1 *in vitro* secondary antibody responses of PE⁺IgG1⁺ memory B cells

Memory B cells	CD4 ⁺ T cells	Antibodies (μ g ml ⁻¹)					
		Anti-PE			Anti-NP		
		IgG1	IgM	IgA	IgG1	IgM	IgA
PE ⁺ NP ⁻	PE primed	34.1±1.0	<0.1	33.7±0.1	<0.1	<0.1	<0.1
PE ⁺ NP ⁻	NP/CG primed	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
NP ⁺ PE ⁻	PE primed	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

Numbers represent the concentration of anti-PE or anti-NP antibody (in μ g ml⁻¹). Titres were obtained from three independent wells and the mean ± s.e.m. is shown.

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A common E2F-1 and p73 pathway mediates cell death induced by TCR activation

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Strong stimulation of the T-cell receptor (TCR) on cycling peripheral T cells causes their apoptosis by a process called TCR-activation-induced cell death (TCR-AICD)^{1–3}. TCR-AICD occurs from a late G1 phase cell-cycle check point⁴ independently of the ‘tumour suppressor’ protein p53 (refs 5, 6). Disruption of the gene for the E2F-1 transcription factor^{7,8}, an inducer of apoptosis^{9–11}, causes significant increases in T-cell number and splenomegaly^{12–15}. Here we show that T cells undergoing TCR-AICD induce the p53-related gene p73, another mediator of apoptosis¹⁶, which is hypermethylated in lymphomas^{17,18}. Introducing a dominant-negative E2F-1 protein or a dominant-negative p73 protein into T cells protects them from TCR-mediated apoptosis, whereas dominant-negative E2F-2, E2F-4 or p53 does not. Furthermore, E2F-1-null or p73-null primary T cells do not undergo TCR-mediated apoptosis either. We conclude that TCR-AICD occurs from a late G1 cell-cycle checkpoint that is dependent on both E2F-1 and p73 activities. These observations indicate that, unlike p53, p73 serves to integrate receptor-mediated apoptotic stimuli.

Previously, we showed that TCR-AICD occurs from a late G1 cell-cycle death checkpoint⁴ in a p53-independent manner^{5,6}. However, the mediators of this apoptotic programme remain unknown. To examine this process, we used centrifugally elutriated early G1 phase Jurkat T cells and mimicked TCR engagement by crosslinking the TCR with anti-CD3 antibodies¹⁹. Apoptosis was first detected in

TCR-stimulated early G1 T cells at 6 h after stimulation by trypan blue vital dye exclusion and TdT-mediated dUTP nick end labelling (TUNEL) assay (Fig. 1a). In addition, treatment with cycloheximide, a protein synthesis inhibitor, inhibited TCR-mediated apoptosis (data not shown), indicating that translation and probably transcription were required.

Mice without the gene for the transcription factor E2F-1 suffer from splenomegaly enlarged lymph nodes and lymphomas, potentially owing to a defect in apoptosis^{12,13}. Also, overexpressed ectopic E2F-1 induces apoptosis^{9–11}. In addition, the late G1 cell-cycle position of TCR-mediated apoptosis coincides with transcriptional activation by E2F family members^{7,8}. To examine the involvement of E2F-1 as a mediator of TCR-AICD, we generated transducible TAT-fusion proteins of a dominant-negative E2F-1 protein (residues 1–374) lacking the carboxy-terminal transcriptional transactivation domain (TAT–E2F-1^{DN}), an inactive DNA-binding domain (DBD) mutant (TAT–E2F-1^{ΔDBD}), and a TAT–green fluorescent protein (GFP) fusion as a negative control (Fig. 1b, top). TAT–E2F-1^{DN} protein bound to DNA containing an E2F site *in vitro*, but the TAT–E2F-1^{ΔDBD} protein did not (data not shown). TAT-fusion proteins transduce into about 100% of cells in a rapid, concentration-dependent manner, reaching intracellular equilibrium in less than 15 min^{4,20}, and both TAT–E2F-1^{DN} and TAT–E2F-1^{ΔDBD} fusion proteins were found to transduce into T cells (Fig. 1b, bottom).

TCR-stimulated early G1 phase T cells were treated with 100 nM TAT–E2F-1^{DN}, TAT–E2F-1^{ΔDBD} or TAT–GFP proteins and assayed for apoptosis by TUNEL assay (Fig. 1c) and trypan blue dye exclusion. T cells treated with PBS, TAT–GFP and TAT–E2F-1^{ΔDBD} proteins showed similar high levels of TUNEL-positive genomic DNA at 6 and 9 h after stimulation. In contrast, T cells treated with TAT–E2F-1^{DN} protein were resistant to both DNA fragmentation and apoptosis (Fig. 1c). Furthermore, stimulated T cells treated

Figure 1 TCR-AICD occurs from late G₁ phase and is E2F-1-dependent. **a**, Early G₁ elutriated human Jurkat T cells were TCR (anti-CD3) stimulated and assayed for apoptosis by TUNEL genomic DNA fragmentation. **b**, Diagram of transducible TAT–E2F-1 dominant-negative (TAT–E2F-1^{DN}) and E2F-1 DNA-binding domain (TAT–E2F-1^{ΔDBD}) mutants (top). Anti-HA immunoblot analysis from T cells treated with 100 nM TAT–E2F-1 fusion proteins (bottom). Note that the HA epitope is present in the TAT leader. **c**, Parallel cultures of TCR (anti-CD3)-stimulated (+) or unstimulated (–) early G₁ T cells were treated with 100 nM TAT–E2F-1^{DN}, TAT–E2F-1^{ΔDBD} or control TAT–GFP proteins and assayed for apoptosis at 3, 6 and 9 h after treatment by TUNEL genomic DNA fragmentation. All experiments were performed in duplicate and error bars indicate s.e.m.

with dominant-negative TAT-E2F-2^{DN} and TAT-E2F-4^{DN} proteins were not protected from apoptosis (data not shown). These observations indicate that TCR-AICD is an E2F-1-dependent process.

TCR-AICD is independent of p53 (refs 5, 6, 21), but a p53 family member, p73, induces apoptosis when overexpressed¹⁶. Ectopic

expression of E2F-1 induces p73 messenger RNA levels and apoptosis²². We therefore examined the amount of p73 in TCR-stimulated early G1 T cells over a time course by immunoblot analysis (Fig. 2). p73 levels remained relatively unchanged in control T cells. In contrast, p73 amounts began to increase by 3 h after TCR

Figure 2 TCR-AICD induces p73 protein levels. **a**, Anti-p73 immunoblot analysis from parallel cultures of TCR (anti-CD3) stimulated (+) or unstimulated (-) early G₁ human T cells. p73 α form (relative molecular mass 80,000) is the predominant form observed in

T cells. **b**, Control anti-Cdk2 immunoblot to normalize protein loading. Unstimulated and stimulated T cells contain constant levels of Cdk2 (ref. 4).

Figure 3 TCR-AICD is p73-dependent. **a**, Diagram of transducible TAT-p73 dominant-negative (TAT-p73^{DD}), TAT-p73 mutant (TAT-p73^{MUT}) and TAT-p53 dominant-negative (TAT-p53^{DD}) proteins. **b**, Anti-HA immunoblot analysis from T cells treated with 100 nM TAT-p73 and TAT-p53 fusion proteins. Asterisk, L371P mutation in TAT-p73^{MUT}. **c**, TCR (anti-CD3) stimulated elutriated early G₁ human T cells were treated with 100 nM TAT-p73^{DD}, TAT-p73^{MUT} or control TAT-p53^{DD} proteins and assayed for apoptosis by TUNEL genomic DNA fragmentation at 6 and 9 h after addition. All experiments were performed in duplicate and error bars indicate s.e.m.

Figure 4 Primary T cells require E2F-1 and p73 to undergo TCR-AICD. **a**, TCR (anti-CD3)-stimulated asynchronous, cycling primary wild-type murine peripheral T cells (splenocytes) were treated 100 nM TAT-E2F-1^{DN}, control TAT-E2F-1^{ΔDBD}, TAT-p73^{DD} or control TAT-p73^{MUT} proteins and assayed for apoptosis at 20 h after addition. All experiments were performed in triplicate and error bars indicate s.e.m. **b**, Cycling wild-type, E2F-1-null and p73-null primary T cells (splenocytes) were TCR (anti-CD3) stimulated and assayed for apoptosis at 20 h after stimulation. Error bars indicate s.e.m.

stimulation, slightly before the increase of TUNEL-positive T cells, and are consistent with a recent report²³. To investigate the role of p73 in mediating TCR-AICD, we generated a transducible p73 dominant-negative TAT-fusion protein (TAT-p73^{DD}) corresponding to the tetramerization domain of p73 (residues 329–430) that binds to and interferes with p73 function, but does not bind p53 (refs 22, 24; Fig. 3a). An inactive control TAT-p73^{MUT} mutant protein, corresponding to an inactivating tetramerization point mutation (L371P) found in p53 from Li-Fraumeni patients^{25,26}, and a p53 tetramerization domain dominant-negative (TAT-p53^{DD}) control protein were also generated (Fig. 3a). TAT-p73^{DD}, TAT-p73^{MUT} and TAT-p53^{DD} proteins transduced into cells with near equal efficiency (Fig. 3b). TCR-stimulated early G1 T cells were treated with 100 nM TAT-p73^{DD}, TAT-p73^{MUT} or TAT-p53^{DD} proteins and assayed for apoptosis by TUNEL genomic DNA fragmentation at 6 and 9 h after addition (Fig. 3c). Both TAT-p73^{MUT} and TAT-p53^{DD} proteins failed to protect T cells from apoptosis. In contrast, TCR-stimulated T cells treated with TAT-p73^{DD} protein showed only background levels of TUNEL-positive cells.

To determine further the requirement for E2F-1 and p73 in T-cell apoptosis, we examined anti-CD3-stimulated primary murine peripheral T cells (splenocytes). Consistent with the observations above, stimulated murine splenocytes were also protected from apoptosis by treatment with TAT-E2F-1^{DN} and TAT-p73^{DD} proteins, whereas inactive mutant proteins failed to inhibit apoptosis (Fig. 4a). We next assayed the ability of E2F-1-null^{12,13} and p73-null²⁷ primary splenocytes to undergo apoptosis. Anti-CD3-stimulated wild-type T cells underwent apoptosis consistent with the results above (Fig. 4b). In contrast, both the E2F-1-null and the p73-null cells failed to undergo apoptosis (Fig. 4b). These observations from dominant-negative transducing proteins and genetic deletions show that E2F-1 and p73 are specific mediators of TCR-AICD.

Previous studies have demonstrated that ectopic overexpression of E2F-1 promotes apoptosis^{9–11}, but here we show that TCR-mediated apoptosis requires endogenous levels of E2F-1 to initiate apoptosis in mature peripheral T cells. These observations are consistent with the disruption of lymphatic homeostasis observed in E2F-1-null mice^{12–15}. TCR-AICD is a p53-independent process^{5,6,21}, but the p53-related gene, p73, is both induced in T cells undergoing TCR-AICD²³ and a downstream target of E2F-1 (ref. 22). Consistent with these observations, inactivation of pRB, which is a regulator of E2F-1, by SV40 T antigen or adenoviral E1A also results in increased p73 (refs 28, 29). Moreover, several studies have reported transcriptional silencing of the p73 gene by hypermethylation in human lymphomas, indicating a possible involvement in oncogenesis^{17,18}. Thus, based on the ability of TAT-p73^{DD} protein to block apoptosis and the absence of apoptosis in both E2F-1- and p73-null T cells, we conclude that TCR-mediated apoptosis is dependent on both E2F-1 and p73. These observations indicate that a common pathway mediates TCR-AICD where p73 is a downstream target of E2F-1, rather than E2F-1 induction of an unidentified target gene. However, we cannot exclude the possibility that E2F-1 and p73 have parallel, but obligate pathways to induce apoptosis. Thus, unlike p53, which serves as a checkpoint protein for DNA damage, p73 appears to integrate stimuli for both DNA damage and receptor-mediated apoptosis. □

Methods

Cell culture

Human Jurkat T cells were obtained from ATCC and maintained as previously described⁴. Centrifugal elutriations were performed as described⁴. Murine splenocytes were isolated as described³⁰ and activated by treatment with con-A (2 µg ml⁻¹) and IL-2 (10 U ml⁻¹). For TCR stimulation, elutriated T cells were resuspended in fresh media at 1.5 × 10⁶ cells ml⁻¹ and treated with 4 µg ml⁻¹ of soluble anti-CD3 (HIT3a) antibodies (Pharmingen) and splenocytes were treated with plate crosslinked hamster anti-murine CD3 antibodies (gift from J. Russell) plus IL-2 (10 U ml⁻¹). TAT-fusion proteins were added to T-cell cultures

one hour before stimulation at 100 nM final concentration. Cell viability was determined by TUNEL positive genomic DNA assay (Trevigen) as described by the manufacturer and trypan blue exclusionary dye. The percentage of TUNEL-positive cells was determined by dividing the number of TUNEL-positive staining cells per field by total number of cells. Confocal microscopy of cells treated with fluorescein-isothiocyanate-labelled TAT-fusion proteins was performed as described²⁰.

TAT-fusion protein purification

E2F-1 complementary DNAs (residues 1–374 and 1–374Δ131–184) were cloned into the NcoI–EcoRI site of pTAT-HA vector²⁰. p73 dominant-negative and inactive point mutant (residues 326–430), and p53 dominant-negative (residues 319–393) cDNAs were cloned into the KpnI–EcoRI sites of the pTAT-HA vector. TAT–GFP was generated by inserting the GFP open reading frame into the NcoI and EcoRI sites of pTAT-HA. All TAT-fusion proteins were purified as described²⁰, fractions containing TAT-fusion protein underwent buffer exchange on PD10 columns (Pharmingen) into PBS (TAT–E2F-1) or TE (TAT–p73, p53). Immunoblots were performed with anti-haemagglutinin (anti-HA; Babco), anti-p73 (Oncogene) or anti-Cdk2 (Santa Cruz) antibodies as described²⁰.

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Role for the p53 homologue p73 in E2F-1-induced apoptosis

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The transcription factor E2F-1 induces both cell-cycle progression and, in certain settings, apoptosis. E2F-1 uses both p53-dependent and p53-independent pathways to kill cells^{1–8}. The p53-dependent pathway involves the induction by E2F-1 of the human tumour-suppressor protein p14ARF, which neutralizes HDM2 (human homologue of MDM2) and thereby stabilizes the p53 protein⁹. Here we show that E2F-1 induces the transcription of the p53 homologue p73. Disruption of p73 function inhibited E2F-1-induced apoptosis in p53-defective tumour cells and in p53^{−/−} mouse embryo fibroblasts. We conclude that activation of p73 provides a means for E2F-1 to induce death in the absence of p53.

Figure 1 E2F-1 induces p73 mRNA and protein accumulation. **a**, SAOS2 cells were immunoblotted with an anti-p73 antibody at the indicated times after induction of wild-type (wt) or mutant E2F-1. **b**, Northern blot analysis of SAOS2 cells with radiolabelled p73 and GAPDH cDNA probes at the indicated time points after induction of E2F-1 or E2F-1(132E). Several p73 mRNA transcripts were detected, possibly owing to alternative splicing¹¹.

To determine whether E2F-1 could activate p73, we used SAOS2 osteosarcoma subclones in which wild-type or mutant derivatives of E2F-1 were controlled by an inducible promoter¹⁰. Induction of wild-type E2F-1 led to an increase in p73 protein levels, whereas induction of DNA-binding (E2F-1(132E)) or transactivation (E2F-1(1–374)) defective E2F-1 mutants did not (Fig. 1a). Similarly, p73 levels were increased after transient transfection of SAOS2 cells and C33A cervical carcinoma cells with plasmids encoding wild-type, but not mutant, E2F-1 (data not shown). Note that p73 and the related gene p63 both give rise to several protein isoforms owing to differential splicing¹¹. Both p73α and p73β can induce apoptosis¹¹.

E2F-1 activates the transcription of p14ARF, which neutralizes HDM2 and stabilizes p53 (ref. 9). In contrast, direct induction of p14ARF had no effect on p73 protein levels (data not shown). Furthermore, HDM2 degrades p53 but not p73 (ref. 11). Together, these findings suggested that induction of p73 by E2F-1 was not due to post-transcriptional changes mediated by ARF and HDM2. Indeed, E2F-1 induced the accumulation of p73 messenger RNA (Fig. 1b) and transcriptionally activated the p73 promoter (Fig. 2a). In parallel experiments, E2F-1 did not affect p63 mRNA or protein levels (data not shown) despite the high degree of similarity between p63 and p73 (ref. 11). We found that E2F-1 was the most potent activator of both the p73 promoter (Fig. 2b) and apoptosis (data not shown) among the E2Fs tested. In many systems, E2F-1 is the most potent inducer of apoptosis among the E2F family members¹². Inspection of the p73 promoter revealed at least three potential E2F-binding sites located near the transcription start site (see Supplementary Information). The authenticity of these sites was confirmed by electrophoretic mobility shift assays (data not shown), and their removal by a 5' deletion rendered the p73 promoter unresponsive to E2F-1 (see Supplementary Information). Inactivation of these three E2F sites by site-directed mutagenesis did not fully inactivate the promoter (data not shown), suggesting that the resulting mutant p73 promoter contains at least one non-canonical E2F-binding site or that E2F also has indirect effects on p73 transcription. Detailed characterization of the p73 promoter will be reported elsewhere.

E2F transcriptional activity is modulated by pRB family members and peaks in late G1 or S phase¹². We next measured p73 levels in cells that were synchronized by protocols that affect E2F activity. First, confluent HaCaT keratinocytes were plated at low density in the presence or absence of transforming growth factor (TGF)-β. TGF-β prevents pRB phosphorylation and thus suppresses E2F activity and cell-cycle progression. Likewise, TGF-β inhibited p73

Figure 2 The p73 promoter is E2F-responsive. **a**, **b**, SAOS2 cells were transfected to produce the indicated haemagglutinin (HA)-tagged E2F proteins, along with a luciferase reporter plasmid containing the p73 promoter. Plasmid concentrations: triangle represents 10 ng, 100 ng, 1 μg and 2 μg in **a**, and 100 ng and 1 μg in **b**; '+' represents 2 μg in **a** and 1 μg in **b**. Each E2F protein was produced at comparable levels as determined by anti-HA immunoblot analysis (data not shown). Shown are luciferase values corrected for transfection efficiency ($n = 3$ independent experiments).

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Halocarbons produced by natural oxidation processes during degradation of organic matter

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Volatile halogenated organic compounds (VHOC) play an important role in atmospheric chemical processes—contributing, for example, to stratospheric ozone depletion^{1–4}. For anthropogenic VHOC whose sources are well known⁵, the global atmospheric input can be estimated from industrial production data. Halogenated compounds of natural origin can also contribute significantly to the levels of VHOC in the atmosphere⁶. The oceans have been implicated as one of the main natural sources^{7–10}, where organisms such as macroalgae and microalgae can release large quantities of VHOC to the atmosphere^{11,12}. Some terrestrial sources have also been identified, such as wood-rotting fungi¹³, biomass burning¹⁴ and volcanic emissions¹⁵. Here we report the identification of a different terrestrial source of naturally occurring VHOC. We find that, in soils and sediments, halide ions can be alkylated

during the oxidation of organic matter by an electron acceptor such as Fe(III): sunlight or microbial mediation are not required for these reactions. When the available halide ion is chloride, the reaction products are CH₃Cl, C₂H₅Cl, C₃H₇Cl and C₄H₉Cl. (The corresponding alkyl bromides or alkyl iodides are produced when bromide or iodide are present.) Such abiotic processes could make a significant contribution to the budget of the important atmospheric compounds CH₃Cl, CH₃Br and CH₃I.

In a study of VHOC in surface peat-bog waters containing suspended organic matter, we found that besides the well-known trichloromethane which is attributed to an enzymatic halogenation of organic compounds¹⁸, significant amounts of other halocarbons such as iodomethane, iodoethane, iodopropane and iodobutane were released from organic-rich waters. The water samples displayed conspicuous concentrations of Fe(III) (up to 0.1 mmol l⁻¹), a high iodide content and a pH value of 4.2. Investigations of three different soil samples from anthropogenic unpolluted areas in Western Patagonia/Chile and Hawaii (Table 1) showed partially higher concentrations of CH₃Cl, C₂H₅Cl and CH₃Br in addition to the alkyl iodides. Isolation and identification of VHOC were performed by a headspace gas chromatography technique with electron capture and mass spectrometry detection.

To differentiate between biological or geochemical processes, a part of each sample was dried at 105 °C in order to destroy biological active material, such as microorganisms or enzymes, while the other part was freeze-dried. Both samples, when resuspended in distilled water, showed a similar methyl halide distribution to those we found in the natural untreated samples.

We considered that in soils there should exist an abiotic reaction mechanism that forms alkyl halides. The thermodynamically labile organic matter is oxidized and the redox partner (for example, Fe(III)) is reduced (to Fe(II)). Phenolic moieties of the natural organic matter containing methoxy groups might be oxidized while Fe(III) is reduced. During this process halides are methylated, and the methyl halides formed represent degradation products of oxidized organic matter (Fig. 1). Ethoxylated and propoxylated phenolic structures could be responsible for the occurrence of ethyl and propyl halides.

To verify this hypothesis the following experiments were initiated: (1) three different organic-rich soils (Table 1) from the natural reserve Rotwasser (Rotw.) in the Hessian Odenwald (Germany) were collected and tested for VHOC emission in relation to oxidant concentration and halide ion content; (2) for comparison, the same parameters were measured using 2-methoxyphenol (guaiacol), which represents a natural monomeric constituent of humus.

We suspended dried soil samples in sterile distilled water containing Fe(III) and halide ions. Fe(III) was readily reduced to Fe(II) depending on the organic carbon content of the soil samples (Fig. 2a). The higher the organic carbon content of the soil sample the more Fe(III) was reduced during the same period.

Figure 1 Model for alkyl halide formation by the reaction of Fe(III) and organic matter in the presence of halide ions.

Table 1 Organic carbon, pH, Fe, halogens and halogen ratio in different soil samples

Soil	C _{org}	pH	Fe	Cl	Br	I	Cl:Br:I (mol)
	(% dry wt)		(% dry wt)				
Grassland soil A _h (Rotw.)	6.6	4.96	0.70	187	<5.7	<3.7	
Coniferous forest soil A _h (Rotw.)	14.8	3.01	0.39	233	15.8	6.6	123:2.3:1
Suspended matter from peat water (Rotw.)	28.6	4.18	2.95	676	45.9	23.3	100:3:1
Forest soil O _h /A _h (Hawaii I)	26.3	5.6	1.7	195	33.3	37.3	15:1.4:1
Forest soil A _h (Hawaii II)	13.5	4.2	15.4	236	101	236	2.8:0.6:1
Grassland soil O _h /A _h (Patagonia)	46.9	4.10	0.26	2,900	260	32.9	320:12.5:1

Subscript h indicates soil horizon; C_{org} is organic carbon content; Rotw. indicates Rotwasser, Germany (see text); wt, weight.

VHOC release rates were measured during the reduction of Fe(III) in the presence of halide ions. We identified four different alkyl halides with decreasing release rates in the following order: halomethane > haloethane > 1-halopropane ≥ 1-halobutane. To quantify the alkyl halides we used iodide as the halide source. As a result, iodomethane, iodoethane, 1-iodopropane and 1-iodobutane

were formed in the proportion 19:5:1.5:1 (Fig. 2b). An almost similar proportion (17:4.5:0.5:1) was obtained from untreated peat-bog water.

The influence of Fe(III) concentration on the alkyl halide formation can be inferred from Fig. 3a. When higher amounts of Fe(III) were added to the soil, the alkyl halide emission increased. There was a little production of alkyl halides and Fe(II) even if no Fe(III) was added to the soil. This is probably caused by the presence of Fe(III) as a mineral phase in the form of iron oxides and oxyhydroxides in the soil samples (0.7–3% of dry weight) which could be reductively dissolved by natural organic compounds.

Figure 2 Abiotic reduction of Fe(III) in soils and the production of alkyl iodides in the presence of iodide. **a**, Abiotic reduction of Fe(III) to Fe(II) by different organic-rich soils. 100 mg soil sample (dried at 105 °C) was suspended in 10 ml bidistilled water containing 10 μM dissolved Fe(III) as electron acceptor. The pH in the medium was 3.0 and temperature was 30 °C. Subsamples were filtered (0.45 μm pore filter) and Fe(II) was photometrically analysed as ferrous iron-phenanthroline complex. Three to six replicates were measured for each sample ($n = 3-6$). The standard deviation (s.d.) was in the range 7–31% error (σ). **b**, Alkyl iodide formation of a grassland soil (Rotw.) in the presence of Fe(III) and iodide. 100 mg grassland soil (dried at 105 °C) was added to 10 ml bidistilled water in a 20-ml headspace flask containing 10 μM dissolved Fe(III). Before the flask was sealed the medium (pH 3.0) was supplemented with 100 μM KI. After shaking at a temperature of 30 °C the volatile halogenated organic compounds (VHOC) in the headspace were determined by gas chromatography (GC) with an electron capture detector (ECD) ($n = 4-6$; s.d. 4–25% error).

Figure 3 Influence of Fe(III) and halide ions on the formation of alkyl halides. **a**, Alkyl iodide production from a coniferous forest soil (Rotw.) by using Fe(III) as electron acceptor. Coniferous forest soil (500 mg, dried at 105 °C) was suspended in 10 ml bidistilled water. In 20-ml headspace flasks. The medium (pH 3.0) was supplemented with KI (100 μM) and 5–100 μM Fe(III). The flasks were sealed and shaken for 1 h at a temperature of 30 °C. VHOC concentrations in the headspace were measured by GC–ECD ($n = 4-5$; s.d. 5–44% error). **b**, Effect of halide ion concentration on yield of corresponding halomethane production with a grassland soil (Rotw., from Rotwasser, Germany, see text) as organic matter source. 1,000 mg grassland soil (freeze-dried) was added to 10 ml bidistilled water in a 20-ml headspace flask. The medium (pH 5.0) was supplemented with 1–10 mM KCl, KBr or KI before the flask was sealed. After shaking for 60 min at a temperature of 30 °C, halomethane concentration in the headspace was monitored by GC–ECD ($n = 4-6$; s.d. 7–25% error).

Figure 3b illustrates the effect of different halide concentrations on the yield of volatile halomethanes with a grassland soil sample (Rotw.) as the organic matter source. Halomethane production increased with increasing halide ion concentration. When equimolar concentrations (1 mM) of chloride, bromide and iodide were present, the preferred halide ion was iodide, the respective halomethanes being formed in the proportion of 1:1.5:10. We applied this experimentally determined proportion to the natural molar halogen ratios of the Patagonian soil (Cl:Br:I; 320:12.5:1), the Hawaiian soils I (15:1.4:1) and II (2.8:0.6:1), to obtain $\text{CH}_3\text{Cl}:\text{CH}_3\text{Br}:\text{CH}_3\text{I}$ ratios of 32:1.8:1, 7:1:5 and 3:1:11, respectively. Very similar ratios of 48:1.9:1, 5.5:1:2.9 and 3.5:1:8.7 were measured from these natural untreated samples. Our results suggest that a fraction of the soil halogen content is methylated by natural oxidation processes and that this fraction increases in the sequence $\text{Cl} < \text{Br} < \text{I}$.

The formation of methyl halides by a model reaction of guaiacol with ferrihydrite ($5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$) and halide is presented in Fig. 4. These findings are in agreement with the data we presented from natural soils. Ferrihydrite, the most common initial precipitate of

iron oxyhydroxides in nature, was used to oxidize guaiacol. Methyl halide, Fe(II) and *o*-quinone have been identified as reaction products.

There was no production of halomethanes if ferrihydrite or halide was absent. We assume that methyl halides are produced in an almost synchronous reaction scheme: (1) the oxidation of guaiacol by ferrihydrite and (2) nucleophilic substitution of the methyl group by halide.

The low molar ratio of halomethane to Fe(II) (Fig. 4a) indicates that further oxidation products of guaiacol were produced. The formation of a methoxyphenol dimer as a result of oxidative oligomerization of clays has been reported¹⁷. However, our reaction model describes a chemical pathway leading from guaiacol to methyl halide. This reaction is specific only for the methyl halides but in additional experiments with ethoxyphenol and propoxyphenol the corresponding ethyl halides and propyl halides were found.

Soil is a highly dynamic and complex system where a multitude of biological, bio-mediated and abiotic processes interact. In this study we have shown that in soils and sediments there exists an abiotic process, hitherto unknown, forming alkyl halides, which depends on organic-matter content, halide-ion and oxidant concentrations. These findings may have important implications for the global production of VHOC. For example, it is known that: (1) the reduction of insoluble Fe(III) oxides and oxyhydroxides is one of the most significant geochemical processes that takes place in the sedimentary environment¹⁸; (2) many terrestrial soils exhibit high salinities (up to 1% of dry weight); (3) worldwide 1,500–2,200 Gt of organic carbon is stored as humus in the soil layers¹⁹, and alkoxy-phenolic structures, especially methoxylated phenolic structures, are monomeric constituents of humic substances. Nevertheless, the magnitude of VHOC production in soil is difficult to estimate. In our model experiments, a maximum of 1 of 30,000 carbon atoms was liberated from the soil as VHOC within one hour. We cannot use this relationship to estimate a global emission rate, but our results do show that the terrestrial ecosystem has an enormous potential to release CH_3Cl , CH_3Br and CH_3I into the atmosphere.

The described processes will depend on many factors, including the ambient environmental conditions (such as moisture, temperature and soil acidity) and the type of organic material within the soil. Additional studies and associated field measurements are required in order to calculate the release rates of VHOC from soils and sediments, and to distinguish further between biogenic and abiotic production. □

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Figure 4 Data supporting the abiotic production of methyl halides by the interaction of guaiacol with ferrihydrite and halide. **a**, Reductive dissolution of ferrihydrite by guaiacol (2-methoxyphenol) and the related iodomethane formation. 20 mg ferrihydrite and 10 mM KI were suspended in 10 ml bidistilled water in a 20-ml headspace flask under argon atmosphere. The pH of the medium was adjusted with 1 M HNO_3 to a value of 4.4. Guaiacol (2 mM) was added and the flask was sealed. Subsamples were taken for Fe(II) and iodomethane detection ($n = 3$ –6; s.d. 9–22% error). **b**, Influence of ferrihydrite on yield of halomethane production. 10 mM KCl, KBr or KI and 5–275 mg ferrihydrite were added to 10 ml bidistilled water in a 20-ml headspace flask. The pH of the medium was adjusted with 1 M HNO_3 to a value of 4.4. Before the flask was sealed the medium was supplemented with 2 mM guaiacol. After a reaction time of 60 min methyl halide was monitored ($n = 3$ –6; s.d. 4–32% error).

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Annual fluxes of carbon from deforestation and regrowth in the Brazilian Amazon

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The distribution of sources and sinks of carbon among the world's ecosystems is uncertain. Some analyses show northern mid-latitude lands to be a large sink, whereas the tropics are a net source¹; other analyses show the tropics to be nearly neutral, whereas northern mid-latitudes are a small sink^{2,3}. Here we show that the annual flux of carbon from deforestation and abandonment of agricultural lands in the Brazilian Amazon was a source of about 0.2 Pg C yr⁻¹ over the period 1989–1998 (1 Pg is 10¹⁵ g). This estimate is based on annual rates of deforestation and spatially detailed estimates of deforestation, regrowing forests and biomass. Logging may add another 5–10% to this estimate⁴, and fires may double the magnitude of the source in years following a drought⁴. The annual source of carbon from land-use change and fire approximately offsets the sink calculated for natural ecosystems in the region^{5,6}. Thus this large area of tropical forest is

nearly balanced with respect to carbon, but has an interannual variability of ± 0.2 PgC yr⁻¹.

We determined the annual flux of carbon with a 'bookkeeping' model^{7,8} that tracks the annual emission and uptake of carbon that follow the clearing of forest for agriculture and the regrowth of secondary forests on abandoned agricultural land. Changes in carbon include (1) the immediate loss of carbon to the atmosphere from plant material burned at the time of clearing, (2) the slower release of carbon from decay of dead plant material left on site (slash) and removed for wood products, and (3) the accumulation of carbon during forest growth. Changes in soil carbon were not included in this analysis, as they are small relative to the changes in biomass and are inconsistent in direction^{9–12}.

We used two estimates of deforestation, three estimates of biomass and two estimates of the rate of decay of organic matter to calculate a range of net carbon emissions attributable to land-use change. The first estimate of deforestation was obtained from the Brazilian Space Agency (INPE), where data from the Landsat satellite are delineated manually for each state to determine both annual rates of deforestation and cumulative areas deforested for each year between 1988 and 1998 (except 1993). The annual and cumulative data are not entirely consistent, and we used the cumulative areas deforested to calculate annual rates of change (Table 1). INPE also determined the area deforested in 1978; before 1960 rates of deforestation were negligible¹³.

The second estimate of deforestation was based on a map of land cover derived from classification of 1986 Landsat multi-spectral scanner data (Fig. 1). Areas classified as deforested in 1986 were consistently lower than INPE's 1988 estimate of deforested area. Because the dates were different, we interpolated a rate for 1988 based on maps of land cover derived from 1986 and 1992 Landsat data. The interpolated area deforested in 1988 was still about 25% lower than INPE's estimate, although the actual percentage varied among states (Fig. 2). We used this lower estimate for a second estimate of deforestation, varying it annually in proportion to the rates from INPE.

According to the Landsat-derived land-cover classification, about 30% of the deforested area was in secondary forest in 1986—presumably as a result of the abandonment of agricultural land^{14–17}. The percentage varied from 5% in Goiás to 65% in Maranhão. As we lacked data to suggest temporal trends in abandonment, we assumed that cleared lands were abandoned annually at the rate defined by the ratio of secondary forest to deforested area in 1986.

Figure 1 Land cover in Brazilian Amazonia as of 1986, based on a classification of Landsat MSS data. The classification identifies seven classes of land cover: forest, deforested land, regrowing forest, water, clouds, cloud shadow and *cerrado* (savanna). Here data for cloud and cloud shadow are grouped together.